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PERFORMANCE AND HAEMATOLOGICAL PARAMETERS OF GROWING RABBITS FED DIFFERENT LEVELS OF VITAMIN E INCLUSION

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Abstract

Rabbit production is an attractive proposition for the supply of high quality meat especially in developing countries with little cereal surplus (Fayeye and Ayorinde, 2008). They are efficient converters of feed to meat. Thus, this study aimed to examine effects of vitamin E inclusion levels on performance and haematological parameters of growing rabbits. A total of forty eight (48) weaner rabbits of mixed breeds and sexes were randomly assigned into four treatments of twelve rabbits each. These rabbits were housed three (3) per cell. The rabbits were placed on four levels of vitamin E inclusion (0mg/kg {Control}, 100mg/kg, 200mg/kg and 300mg/kg) in a completely randomized design. Data were collected on the growth, haematological and serum biochemistry of the growing rabbits. Results obtained showed that levels of vitamin E inclusion significantly ($P<0.05$) influenced final weight, weight gain, total feed intake and feed conversion ratio of growing rabbits. Highest final weight and weight gain was obtained at 300mg/kg of vitamin E inclusion which differed significantly from other dietary treatment groups. Total feed intake was statistically higher in growing rabbits fed 100mg/kg of vitamin E inclusion which differed significantly ($P>0.05$) from values obtained at 200mg/kg and 300mg/kg inclusion levels. Best feed conversion ratio was obtained at 300mg/kg of vitamin E inclusion which differed significantly from other dietary treatments. Results obtained on haematological and serum biochemistry showed that levels of vitamin E inclusion did not significantly ($P>0.05$) influenced all the parameters measured. However, all the values obtained in this study on blood parameters are within the ranges of values reported as baseline data in growing rabbits by authors. It can be concluded that inclusion of dietary vitamin E at 300mg/kg in the diets of growing rabbits could help in enhancing growth performance of growing rabbits. Inclusion of dietary vitamin E in the diet of growing rabbits could help in improving immune responsiveness in growing rabbits has the result obtained in this study does not have any adverse effect on the haematological and serum biochemistry of growing rabbits.

Keywords: Rabbits; growth; Vitamin E and haematology

PARAMETRES DE PERFORMANCE ET D'HEMATOLOGIE DES LAPINS EN CROISSANCE SOUMIS AUX REGIMES CONTENANT DIFFERENTS NIVEAUX D'INCLUSION DE VITAMINE E

Resume

La production de lapins est une proposition attrayante pour l'approvisionnement en viande de haute qualité, en particulier dans les pays en développement ayant un faible surplus en céréales (Fayeye et Ayorinde, 2008). Les lapins sont des convertisseurs efficaces d'aliments animaux en viande. Ainsi, la présente étude avait pour objectif d'examiner les effets de différents niveaux d'inclusion de vitamine E sur les paramètres de performance et d'hématologie des lapins en croissance. Au total, quarante-huit (48) lapins sevrés de races mixtes et des deux sexes ont été répartis de manière aléatoire en quatre traitements de douze lapins par traitement. Ces lapins ont été logés par groupe de trois (3) par cellule. Les lapins ont été soumis à quatre niveaux d'inclusion de vitamine E (0 mg / kg {Control}, 100 mg / kg, 200 mg / kg et 300 mg / kg) selon un schéma complètement randomisé. Des données ont été recueillies sur la croissance, l'hématologie et la biochimie sérique des lapins en croissance. Les résultats obtenus ont montré que les niveaux d'inclusion de vitamine E ont significativement ($P <0,05$) influencé le poids final, le gain pondéral, la quantité totale absorbée et l'indice de consommation des lapins en croissance. Les niveaux les plus élevés

de poids final et de gain pondéral ont été obtenus pour le taux d'inclusion de vitamine E de 300 mg / kg, qui différait significativement des autres groupes de traitement alimentaire. La quantité totale absorbée était statistiquement plus élevée chez les lapins en croissance soumis au taux d'inclusion de vitamine E de 100 mg / kg qui différait significativement (P> 0,05) des valeurs obtenues aux niveaux d'inclusion de 200 mg / kg et de 300 mg / kg. Le meilleur indice de consommation a été noté pour le niveau d'inclusion de vitamine E de 300 mg / kg qui était significativement différent des autres traitements diététiques. Les résultats obtenus pour la biochimie hématologique et sérique ont montré que les niveaux d'inclusion de vitamine E n'ont pas influencé significativement (P> 0,05) tous les paramètres mesurés. Cependant, dans cette étude, toutes les valeurs obtenues sur les paramètres sanguins se situent dans les fourchettes des valeurs rapportées par les auteurs comme données de référence chez les lapins en croissance. On peut conclure que l'inclusion de vitamine E alimentaire à 300 mg / kg dans le régime des lapins en croissance peut contribuer à l'amélioration des performances de croissance des jeunes lapins. L'inclusion de vitamine E dans le régime alimentaire des lapins en croissance pourrait contribuer à améliorer la réponse immunitaire des lapins en croissance, car le résultat obtenu dans cette étude a montré que la vitamine E n'a aucun effet négatif sur la biochimie hématologique et sérique des lapins en croissance.

Mots-clés : lapins ; croissance; vitamine E et hématologie

Introduction

The shortage of animal protein in the developing countries in the tropics has been long recognized and has remained one of the major limiting factors to the attainment of food security in Nigeria. The minimum protein requirement is estimated at about 75g/person/day out of which 40grams should come from animal protein (Akinwumi, 2011). Presently, animal protein consumption has been given as 7g/person/day by FAO (2014), which suggests a less than 16% contribution of the animal protein consumption. In recent years, the consumers have become increasingly interested in the beneficial health-promoting effects of functional foods enriched with natural ingredients such as rabbit meat; these meat are good sources of minerals, vitamins and fatty acids which can be further enriched via feeding (Dalle Zotte and Szendro, 2011; Marounek *et al.,* 2009).

Haemtological studies are useful in the diagnosis of many diseases as well as investigation of the extent of damage to blood (Onyeyili *et al.,* 1992; Togun *et al.,* 2007). Haematological indices are factors that help in investigating the presence of several metabolites and other constituents in the body of animals and it plays a vital role in the physiological and pathological status of an organism (Aderemi, 2004).

Vitamin E (□-tocopherol) is an important antioxidants that cannot be synthesized by most mammals and humans and therefore are required from the diet. Vitamin E, a dietary essential, fat-soluble vitamin, can enhance animal performance when provided in amounts above minimal requirements (Roger, 1999). Vitamin E is essential for body functions such as growth, immune function enhancement, tissue integrity, reproduction and disease prevention (Dalle Zotte and Szendro,2011; Rooke *et al.,* 2004).

In the rabbit protection against peroxide damage depends on vitamin E rather than on selenium (Cheeke, 1987) because the kidneys and liver have a high level of non-selenium dependent *glutatione peroxidase* activity (Lee *et al.,* 1979), hence the vitamin E requirement for rabbits is higher than for ruminants (Jenkins *et al.,* 1970). Intake of vitamin E above the daily requirement increased □-tocopherol levels in plasma and tissues, thus improving lipid stability (Lin *et al.,* 1989; Liu *et al.,* 1995). Thus, this study aims to evaluate, the effect of vitamin E inclusion at different levels on performance and haematological parameters of weaner rabbits since higher inclusion levels have not been reported to the toxic in the diet of growing rabbits

Materials and Methods

The experiment was carried out at the rabbitary Unit of the Directorate of University

Farms, Federal University of Agriculture, Abeokuta, Ogun State. The site is located in the rain forest vegetation zone of South-Western Nigeria on latitude 7o 13' 49.46" N, longitude 3o 26 11.98E and altitude 76m above the sea level. The climate is humid with a mean annual rainfall of 1037mm and mean temperature and humidity of 34.70C and 83%, respectively (Google Earth, 2015).

Experimental Animals and Management

A total of forty- Eight (48), 5 weeks old weaner rabbits of mixed breeds and sexes were used for the study. The rabbits were quarantined for 1 week and raised in individual cages. The rabbits were weighed and balanced for sexes before the commencement of the experiment, the initial live weight of the weaner rabbits ranged from 500 – 510 grams. Feeders and drinkers were provided for ad libitum feeding and watering respectively.

Experimental Design

The forty eight weaner rabbits were exposed to four levels of vitamin E inclusion which were (0mg/kg vitamin E which serves as the control diet, 100mg/kg vitamin E, 200mg/kg vitamin E and 300mg/kg vitamin E). The rabbits were divided into four dietary treatments of twelve rabbits each. These rabbits were housed three (3) per cell in hutches that was washed and disinfected prior to the commencement of experiment. The rabbits were placed on the experimental diet at different levels of vitamin E inclusion in a completely randomized design. The experiment lasted for a period of 10 weeks. The composition of the experimental diet fed to the weaner rabbits is shown in table 1

Treatment 1 (T1) – 0 mg/kg Vitamin E inclusion (Control)

Treatment 2 (T2) – 100 mg/kg Vitamin E inclusion

Treatment 3 (T3) – 200 mg/kg Vitamin E inclusion

Treatment 4 (T4) – 300 mg/kg Vitamin E inclusion

Data Collection

The experiment lasted for 10 weeks, (70days) during which data were collected on the feed intake, changes in weight of the growing rabbits and feed conversion ratio.

Feed intake per rabbit/ day

The rabbit feed intake measures the amount of feed consumed per rabbit/day. It was expressed thus as:Feed intake =feed offered – feed left.

Table 1: Composition of concentrate diet (% as fed)

Ingredients (%)	T1	T2	T3	T4
Maize	48.00	48.00	48.00	48.00
Fish meal	2.00	2.00	2.00	2.00
Soybean meal	3.00	3.00	3.00	3.00
Wheat offal	10.00	10.00	10.00	10.00
Groundnut cake	14.00	14.00	14.00	14.00
Rice husk	20.00	20.00	20.00	20.00
Bone meal	1.50	1.50	1.50	1.50
Oyster shell	1.00	1.00	1.00	1.00
Salt	0.25	0.25	0.25	0.25
Vitamin and Mineral premix	0.25	0.25	0.25	0.25
Total	100	100	100	100
*Vitamin E inclusion	0.00	0.01	0.02	0.03

Determined Analysis	
ME (Kcal/kg)	2591.80
Ash (%)	2.74
Crude fibre %	15.50
Crude protein	15.80
Nitrogen free extract	40.50
Crude protein	15.80
Nitrogen free extract	40.50

Note: *premix:Vit A 8000 iu,Vit D3 2000 iu,Vit E 3000 iu,Vit K 2 mg, Riboflavin 4.20 mg,Vit B12 0.01 mg, Pantothenic acid 5 mg, Nicotinic acid 20 mg, Folic acid 5 mg, Choline 300 g, Mn 56 mg, Fe 20 mg, Cu 10 mg, Zn 50 mg.*

Weight gain - This was measured on weekly basis for a period of 10 weeks by subtracting the initial weight from the final weight

Weight Gain (g/rabbit)=Final Live weight-Initial Liveweight

Feed conversion ratio of the weaned rabbit (FCR)

This was expressed as feed intake in grams divided by the weight gain.

$$\text{Feed conversion ratio} = \frac{\text{Average Feed Intake (g/rabbit)}}{\text{Average Daily Weight Gain (g/rabbit)}}$$

Blood Collection

Blood samples were collected on the 70th day of the experiment.At the time of blood collection eight grower rabbits were selected from each treatment for blood collection. Blood sample of 3ml was withdrawn from the ear vein of each rabbit by means of sterile hypodermic needle and syringe, 2ml of blood collected was released into labeled sample bottles containing ethylene diamine tetra acetate (EDTA) to prevent blood coagulation and was taken to the laboratory while the remaining 1ml was put into bottles without coagulation for blood serum analysis. The following parameters were determined: Packed cell volume (PCV), *haemoglobin* concentration (Hb), white blood cells differentials, red blood cells (RBC), serum total protein, globulin and albumin. Blood parameters were analyzed as described by Jain (1993). Red blood cells (RBC) count was determined using the *Neubauer haemocytometer* after appropriate dilution (Lamb, 1981).

Statistical Analysis

All data obtained were subjected to one way analysis of Variance (ANOVA) (SAS 1999) and significant difference between treatments means were separated using Duncan's multiple range test of the same statistical package.

Results and Discussion

The effect of different levels of vitamin E inclusion on performance of growing rabbits is shown in table 2.Vitamin E at different levels of inclusion significantly ($P<0.05$) influenced final weight, weight gain, total feed intake and feed conversion ratio. The result shows that final weight was highest from growing rabbits fed 300mg/kg vitamin E inclusion level. This result showed that vitamin E inclusion in the diet of growing rabbits improved growth performance and weight gain of the rabbits in this group compared to other dietary treatment groups. This result is similar to what was reported by El-Medany et al.(2012)who found higher final weight in growing rabbits supplemented higher dosage of vitamin E inclusion in their diet compared to other dietary treatments. The results obtained on weight gain and feed conversion ratio showed that highest weight gain and best feed conversion ratio was obtained at 300mg/kg vitamin E inclusion levels which differed significantly from other levels of vitamin E inclusion. This result further supports the findings of El-Medany et al. (2012) who found higher live weight gain and better feed conversion ratio in rabbits supplemented higher dosage of vitamin E inclusion compared

Table 2: Performance of growing rabbits fed different levels of vitamin E inclusion.

Parameters	0mg Vit.E	100mg Vit.E	200mg Vit.E	300mg Vit.E	S.E.M
Initial weight (g)	505.35	510.25	507.10	505.75	10.25
Final weight (g)	1341.25^{c}	1340.50^{c}	1377.30^{b}	1420.00^{a}	20.15
Weight gain (g)	835.90^{c}	830.25^{c}	870.20^{b}	914.25^{a}	10.45
Total feed intake (g)	4133.50^{ab}	4210.50^{a}	4077.50^{b}	4096.40^{b}	40.25
Av./wt./gain/day	11.94	11.86	12.43	13.06	0.81
Feed conversion ratio	4.95^{c}	5.07^{a}	4.68^{c}	4.48^{b}	0.55
Feed intake/rabbit/day	59.05	60.15	58.25	58.52	0.95

$^{a, b, c}$:Means in the same row with different superscripts differ significantly (p<0.05)
SEM: Standard error of mean

Table 3: Haematological and serum biochemistry of growing rabbits fed different levels of vitamin E inclusion.

Parameters	0mg Vit.E	100mg Vit.E	200mg Vit.E	300mg Vit.E	S.E.M
PCV (%)	34.00	32.42	33.52	32.44	1.80
Hb (%)	10.25	10.55	10.29	11.10	0.64
RBC (× 1012l)	6.55	6.61	6.45	6.75	0.73
WBC (× 109l)	7.35	7.45	7.67	7.65	0.89
Neutrophils (%)	28.33	28.37	29.92	30.00	3.54
Lymphocytes (%)	70.55	69.55	70.52	70.53	3.52
Eosinophils (%)	0.15	0.10	0.15	0.25	0.72
Basophils (%)	0.50	0.60	0.64	0.65	0.31
Monocytes (%)	1.10	1.13	1.15	1.14	1.12
*T.Protein (g/dl)	5.65	5.58	5.75	5.80	0.20
Albumin (g/dl)	3.16	3.15	3.09	3.17	0.56
Globulin (g/dl)	2.65	2.52	2.59	2.65	0.48

**T. Protein- Total Protein*

to other treatment groups. Total feed intake differed significantly (P<0.05) across the dietary treatment groups. Feed intake decreases at higher levels (200mg/kg and 300mg/kg) of vitamin E inclusion. Highest feed intake was obtained at 100mg/kg inclusion of vitamin E which differed significantly from other dietary treatments. The result obtained on feed intake in this study disagrees with the findings of Abdel-Khalek et al. (2008) who found higher feed intake at higher inclusion levels of vitamin E in doe rabbit during pregnancy.

The effect of different levels of vitamin E inclusion on *heamatological* parameters and serum biochemistry of growing rabbits is shown in table 3.Vitamin E inclusion at different levels of inclusion did not significantly (P>0.05) influenced all the parameters measured. The results obtained in this study on haematological parameters for all the levels of vitamin E inclusion though not significant (P>0.05) is within the ranges of baseline values reported by Research Animal Resources (2009) in growing rabbits. The results obtained on total protein and albumin in this study though not significant (P>0.05) is similar with the findings Ebeid et al. (2013) who found no significant difference (P>0.05) in growing rabbits fed vitamin E fortified diets. The results obtained on globulin in this study disagrees with the findings of Ebeid et al. (2013) who reported significant (P<0.05) difference in growing rabbits fed vitamin E fortified diets. All the results obtained in this study on serum biochemistry are within the

ranges of values reported by the author above in growing rabbits fed vitamin E fortified diets.

Conflict of Interest

The author(s) declare that there was no conflict of interest.

Conclusion

Based on the result presented above and taking into account vitamin E inclusion at different levels in the diet, it can be concluded that inclusion of dietary vitamin E at 300mg/kg in the diets of growing rabbits could help in enhancing growth performance of growing rabbits. Also inclusion of vitamin E in the diets of growing rabbits has no negative effect on haematology and serum biochemistry of growing rabbits. Furthermore inclusion of vitamin E in the diet of growing rabbits could help in improving immune responsiveness in growing rabbits has the result obtained in this study pose no health challenge to the growing rabbits.

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MANIFESTATION OF CONTAGIOUS BOVINE PLEUROPNEUMONIA IN SEVEN MONTH-OLD CALVES INFECTED VIA BRONCHOSCOPE AND IN CONTACT TRANSMISSION.

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Abstract

Mycoplasma mycoides subsp. *mycoides* (Mmm) causes contagious bovine pleuropneumonia (CBPP) in adult cattle however calves younger than 6 months develop mostly arthritis and associated lameness, but very few lesions in the lungs, suggesting that *Mycoplasma* does not easily colonize lungs of young calves. The aim of this study was to assess effects of CBPP in 7-month-old calves following infection by in-contact transmission; and manifestation in adult cattle and calves infected via intubation method. Both adult and seven month old calves developed respiratory indications of CBPP. Although none of the adult cattle developed arthritis, 7/10 and all 5 calves infected by intubation and in-contact transmission methods respectively developed arthritis. The results show that 7-month-old calves, whether infected by intubation or in-contact method develop respiratory signs similar to adult cattle as well as arthritis as seen in calves younger than six months. This suggests that at seven months, calves have developed susceptibility to lung lesions but still have a propensity for damage in the joints like very young calves.

Key words: Contagious bovine pleuropneumonia, *Mycoplasma mycoides* subsp. mycoides, Calves, intubation, in-contact transmission

MANIFESTATION DE LA PLEUROPNEUMONIE CONTAGIEUSE BOVINE CHEZ DES VEAUX DE SEPT MOIS INFECTES AVEC UN BRONCHOSCOPE ET PAR CONTACT

Resume

Mycoplasma mycoides sous-espèce *mycoides* (Mmm) est responsable de la pleuropneumonie contagieuse bovine (PPCB) chez les bovins adultes, mais les veaux de moins de 6 mois développent essentiellement de l'arthrite et de la boiterie associée, mais très peu de lésions pulmonaires, ce qui porte à croire que *Mycoplasma* ne colonise pas facilement les poumons des jeunes veaux. Le but de cette étude était d'évaluer les effets de la PPCB chez les veaux de 7 mois infectés par contact, et les manifestations chez les bovins adultes et les veaux infectés par intubation. Les veaux adultes et ceux de sept mois ont développé des signes respiratoires de PPCB. Bien qu'aucun des bovins adultes n'ait développé de l'arthrite, 7/10 et tous les 5 veaux infectés par intubation et transmission par contact ont respectivement développé de l'arthrite. Les résultats montrent que les veaux de 7 mois, qu'ils soient infectés par intubation ou par contact, développent des signes respiratoires similaires à ceux des bovins adultes, tels que l'arthrite chez les veaux de moins de six mois. Ceci donne à penser qu'à sept mois les veaux ont développé une susceptibilité aux lésions pulmonaires, mais ont toujours une propension d'endommagement des articulations comme les très jeunes veaux.

Mots-clés : pleuropneumonie contagieuse bovine, *Mycoplasma mycoides* sous-espèce mycoides, veaux, intubation, transmission par contact

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Introduction

Contagious bovine pleuropneumonia is a disease of cattle causing severe pneumonia in adult cattle (≥1 year) characterized by thoracic lesions, especially hepatisation of the lungs and pleurisy (OIE, 2014). On the other hand, calves develop arthritis and associated lameness after infection with *Mmm* (Masiga et al, 1996).

In order to understand various aspects of CBPP as pertains to the host; several infection experiments of cattle by in-contact transmission have been done to simulate natural conditions (Huebschle et al 2006; Totte et al 2010). However this method has several disadvantages because it is expensive, time consuming, and has variable transmission times and infection rates (Dedieu *et al.,* 2005) that make comparison of disease kinetics difficult. To overcome the above drawbacks, an infection model using a bronchoscope was developed (Nkando *et al.,* 2010; Nicholas *et al.,* 2004). This method ensures the delivery of a pure *Mycoplasma mycoides* subsp. myciodes (Mmm) culture and allows the simultaneous infection of experimental animals (Nkando *et al.,* 2010). Several intubation experiments with adult cattle have been shown to reproduce lesions similar to those observed by in-contact transmission (Nkando *et al.,* 2010; Nicholas *et al.,* 2004), although the observed lesions are smaller after intubation than after an in-contact infection, probably as a result of differences between *Mycoplasma* from culture or secreted nasal droplets. Another possible explanation is that intubation of cattle may result in chronic disease patterns than natural exposure (Scacchia et al, 2011). In a previous experiment 2 month old calves infected by in-contact transmission presented with arthritis and associated lameness, and also developed much less severe lung lesions than adult cattle (Mbithi and Wesonga, unpublished data, Sup table 1). In this study, we assessed manifestation of CBPP in 7-month-old calves following infection through in-contact transmission; and manifestation in adult cattle and calves infected via intubation method.

Materials and Methods

Animals

Ten adult (2-3 years old) and 15 three-month-old East African Zebu cattle were purchased from Kakamega district, Western Kenya, a place historically known to be CBPP free. The animals were ear-tagged, bled and tested for CBPP using slide agglutination test (SAT) and complement fixation test (CFT) to confirm their disease free status before purchase. All CBPP negative animals for both SAT and CFT were transported to the experimental research station (Veterinary Research Institute -Kenya Agricultural and Livestock Research Organization (KALRO), Muguga) where they were dewormed with Nilzan plus cobalt® (Cooper, Nairobi, Kenya), and vaccinated against foot-and- mouth disease, lumpy skin disease, black quarter, and anthrax over a period of three months. One month prior to infection, animals were bled weekly for preparation of pre-infection serum samples. All experiments were carried out with permission from the institute's animal care and use committee, under agreement number KARI/VRC/IACUC/2/00122010

Preparation of Mmm infection culture

This was carried out as previously described (Nkando et al, 2010). Briefly, a five ml aliquot of frozen Mmm strain B237 was thawed for 30 min at room temperature and 10-fold dilutions made into bijou bottles containing prewarmed Gourlay broth (Gourlay and Howard 1982). Fifty microliters of the diluted cultures were plated on to Gourlay agar plates and all samples were incubated at 37°C for 48 h in a humidified incubator containing 5% CO2. The bacterial cultures were scaled up into 500 ml and growth monitored daily for turbidity and colour change

Infection of cattle by intubation and in-contact transmission

The cattle were divided into 2 experimental groups; the first group was infected via intubation (10 adult and 10 calves) and the second infected via in-contact

transmission (5 calves). While in a crush, animals were sedated with *xylazine* hydrochloride (Rompun®) (Coopers, Kenya) according to estimated body weight. A bronchoscope (VFS-2A, Swiss Precision, USA) was passed through the nostrils to the larynx and down to the trachea. Sixty (60 ml) of Mmm culture (approximately 109 CCU/ml) was introduced through the bronchoscope into the trachea using a syringe, followed by 15 ml of 1.5% agar suspended in distilled water and finally 30 ml of phosphate buffered saline (PBS) to flush down all of the inoculum (Nkando *et al.,* 2010). After intubation, the cattle infected via in-contact transmission were enclosed together with the former group in a brick house to simulate the natural route of transmission.

Clinical examination

Major clinical observations for each animal were recorded daily at 8:00 AM including rectal temperature, presence of a cough, laboured breathing, and swollen joints. Other signs including teeth grinding, lachrymation, dullness, staggering gait, weakness, nasal discharge, snoring, mouth frothing and shivering were also recorded. Observations on feeding behaviour and limping were made between 9:00 A.M. and 3:30 P.M. when animals where grazed outside.

Serological examination

During the course of the experiment, animals were bled once weekly and the serum samples stored at -20°C until the end of the experiment. The sera were tested for the presence of antibodies against Mmm using complement fixation test (CFT) (Campbell and Turner 1953).

Autopsy, pathology scoring and bacteriological examination

Forty nine days after intubation, cattle were euthanized by captive bolt and then exsanguinated. The carcass was opened along the posterior midline and a sample of pleural fluid, when present, was aspirated into a 10-ml syringe and immediately stored in a cool box. Examination of lungs for gross lesions included measuring and recording lesion type and size. All animals were examined for gross pathological lesions including consolidation, red and gray hepatization, sequestration, and pleuritis. A piece of lung from between the lesion and the grossly normal lung was cut out and placed in a sterile polythene bag, transferred to a cool box, and carried to the laboratory where they were either immediately processed for culturing or stored at □20°C until processing.

The piece of lung was cut into small pieces and placed into bijou bottles containing Gourlay broth (with penicillin and thallium acetate) and incubated at 37°C. After 24 hours, 1 ml of the suspension was titrated out in a 10-fold dilutions series (from 10□1 to 10□3) and 0.2 ml plated onto Gourlay agar plates. They were incubated at 37°C and checked daily for evidence of growth. The agar plates were examined for morphological features and typical "fried egg" appearance at days 1, 5, and 10 under inverted microscope for *Mycoplasma* colonies.

Pathology scoring was carried out to determine severity of the disease in an individual animal (Hudson and Turner 1963). Briefly, the presence of only encapsulated, resolving or fibrous lesions or pleural adhesions was rated 1. The presence of other types of lesions like consolidation, necrosis or sequestration was rated 2. If in addition Mmm was isolated, a 2 was added to the above rating. The resulting score was then multiplied by a factor depending on the lesion size for example, multiply by factor 1 if the lesion size is under 5 cm in diameter, by 2 if it is over 5 cm and under 20 cm, and by 3 if it is over 20 cm in diameter.

Results

The results for adult cattle and calves are summarized in tables 1 and 2 respectively

Most animals in the adult group showed clinical disease as evidenced by fever in 6/10 animals lasting between 1 to 6 days, 9/10 developed cough, and all animals showed at least one or more of the other clinical signs listed above however, none of the adult cattle developed arthritis (Table 1, Supplementary

table 2). Two out of 10 animals sero-converted and antibodies titers of 1:80 and 1:160 to Mmm were detected by CFT. One animal was free from lesions as determined by pathology score, while nine animals showed gross lesions with pathology scores ranging from 1- 6. Mmm was isolated from lungs of 8/10 of the adult animals.

On the other hand, the10 intubated calves showed less severe clinical disease (Table 1) as evidenced by fever in 5/10 animals, with 4 animals showing fever for only 1 day and 1 animal had fever for 5 days; 5/10 developed cough and 7/10 of the calves developed arthritis. All animals showed at least one or more of the other clinical signs (Supplementary table 2). Serologically, 6/10 of the animals sero-converted and antibodies titers to Mmm ranging from 1:10- 1:160 were detected by CFT. Three animals were free from lesions as measured by pathology score, while seven animals showed gross lesions with pathology scores ranging from 1- 6. Mmm was isolated from lungs and/or pleural fluid of 6/10 of the animals (table 1).

In comparison, when the 5 calves were put in-contact with the intubated cattle, 2/5 calves developed cough, sero-converted and had fever lasting one day. All the calves developed arthritis and showed at least one or more of the other clinical signs (Supplementary table 2). Two animals were free from lesions as measured by pathology score, while three calves showed gross lesions with pathology scores ranging 2 and 4. Mmm was cultured from lungs and/or pleural fluid of 3/5 of the animals (Table 1).

Table 1: Duration of fever, pathology scores and highest CFT titers in cattle inoculated via a bronchoscope or in-contact transmission.

Experimental group (Infection method)	Animal no.	Duration of fever (Days)	Clinical signs			Lesions at PM	CFT	Culture results	Pathology score
			Cough	Arthritis	Other				
	842	1	✓	-	✓	Yes	0	+[b]	4
	843	0	✓	-	✓	Yes	0	+[b]	2
	844	0	✓	-	✓	Yes	0	+[b]	6
	990	0	✓	-	✓	Yes	0	+[b]	5
	991	2	✓	-	✓	Yes	1:80	+[b]	4
	992	1	✓	-	✓	Yes	0	+[b]	4
	993	1	✓	-	✓	Yes	0	-	0
	994	2	✓	-	✓	Yes	0	-	1
	995	6	✓	-	✓	Yes	1:160	+[b]	3
	996	0	-	-	✓	Yes	0	+[b]	4
	974	0	✓	✓	✓	Yes	0	+[a]	4
	975	5	✓	✓	✓	Yes	1:40	+[a,b]	4
	976	1	✓	-	✓	Yes	1:40	-	6
	979	0	-	✓	✓	No	1:20	+[a]	2
	981	0	-	-	✓	No	0	+[a,b]	2
	982	0	-	✓	✓	Yes	0	+[a,b]	4
	983	0	-	✓	✓	Yes	0	-	0
	985	1	-	✓	✓	Yes	1:10	-	0

Experimental group (Infection method)	Animal no.	Duration of fever (Days)	Clinical signs			Lesions at PM	CFT	Culture results	Pathology score
			Cough	Arthritis	Other				
	986	1	✓	-	✓	No	1:20	-	0
	987*	1	✓	✓	✓	Yes	1:160	+[a]	6
	977	0	✓	✓	✓	Yes	0	+[a]	4
	978	1	-	✓	✓	Yes	0	+[b]	4
	980	0	✓	✓	✓	No	1:10	+[b]	2
	984	0	-	✓	✓	No	0	-	0
	988	1	-	✓	✓	No	1:10	-	0

*a-Mycoplasma mycoides subsp. mycoides (Mmm) cultured from pleural fluid; b – Mmm cultured from lung; Lesions include one or more of the following not included in the pathology scoring Red and grey hepatization, rubbery appearance, edematous lung lobes, enlarged medistinal lymph nodes and congested kidneys. Other clinical signs include teeth grinding, lachrymation, dullness, staggering gait, weakness, snoring, mouth frothing and shivering. * Animal died 34 days after intubation.*

Table 2: Post mortem findings in experimental cattle infected via bronchoscope and in-contact transmission.

Group	Animal number	Post mortem findings
Adults	842	Both lung lobes with areas of consolidation
	844	Fibrous adhesion between left diaphragmatic lobe and diaphragm (4 ×2 cm); Right diaphragmatic lobe with grey and red hepatization
	994	Left diaphragmatic lobe has resolving lesion; Shiny plasma on both lobes; Rubbery appearance of lungs; Enlarged lymph nodes
	993	Congested kidneys
	843	Congested left kidney
	996	Right cardiac lung surface consolidated
	992	Focal areas of congestion on kidneys; Areas of consolidation on right cardiac and left diaphragmatic surfaces of the lungs; Enlarged mediastinal lymph node
	991	Congested kidneys; Right cardiac and left diaphragmatic surface of the lungs with areas of consolidation
	990	Liver abscess; Fibrous adhesion between left and right diaphragmatic lung surface and the diaphragm; Areas of consolidation on left diaphragmatic surface of lung
	995	Left diaphragmatic lobe has resolving lesion Both lung lobes are edematous
Intubated calves	982	areas of consolidation on right cardiac surface of the lung Arthritis
	974	Approximately 30 ml of pleural fluid , left diaphragmatic surface of lungs
	983	Petechiations on kidneys; Approximately 60 ml pleural fluid Arthritis
	975	Right cardiac surface of lung consolidated; Arthritis
	976	Fibrous adhesion between right cardiac lung surface and thoracic wall (7 cm); Approximately 10 ml pleural fluid
	985	Resolved abscess on spleen; Arthritis

Group	Animal number	Post mortem findings
In contact calves	986	No lesions
	981	No lesions
	979	Arthritis
	980	Arthritis
	977	All lung lobes are edematous; Right cardiac surface of the lung consolidated; Arthritis
	988	Arthritis
	984	Arthritis
	978	Approximately 100 ml of pleural fluid; Right cardiac surface of the lung consolidated; Arthritis

Discussion

The aim of this experiment was to assess the manifestation of CBPP in 7-month-old calves compared to adult cattle, and to compare infection by intubation and in-contact transmission methods in calves.

Eight out of ten of the adult animals infected using a bronchoscope had clinical signs with or without sero-conversion. These results are similar to what is observed in a previous experiment in which 93.75% of the animals infected with a bronchoscope had lesions (Nkando *et al.,* 2010). However the pathology score (measure of severity of disease) was less than that observed in some previous experiments in which maximum scores of 12 were reported (Nkando et al 2010, Mulongo *et al.,* 2015), but similar to mild infections as previously reported (Mwirigi et al, 2016). The difference in our results and those reported by Nkando et al. (2010) and Mulongo et al. (2015) could be attributed to variation between experiments including seasons (dry and rainy), exact age of individual animals and the virulence of infectious material. It could also be that intubation of cattle results in chronic disease patterns when compared to natural exposure (Scacchia et al, 2011)

There was not much difference in the manifestation of CBPP, as measured by the pathology score, in adults and 7-month-old calves. The in-contact group had lower scores, but this is probably due to the fact that the time of infection in such a group can significantly vary and symptoms can be much delayed (Dedieu *et al.,* 2005), thus it is likely that the two animals in this group that showed no lesions, would probably have developed lesions if they had been kept for a longer time. The calves from both groups also developed arthritis similar to what is observed in most calves younger than 6 months (OIE, 2014), in contrast to the adults. Gull *et al.,* 2013 reported that *Mmm* infection in calves resulted in a mild to marked diffuse, subacute, proliferative lymphocytic synovitis. The reason why Mmm causes arthritis in calves, but not adult cattle, is not known, but we can speculate that receptors for Mmm, are expressed in a cell type in the joints of young calves, allowing the *mycoplasma* to adhere and cause inflammation, but those receptors are not expressed or accessible in joints of adult cattle. M. bovis, another cattle *Mycoplasma* causes inflammation in various organs including arthritis and pneumonia in calves and mastitis in adult cattle among others (Pfutzner and Sachse, 1996). In this case, there is no evidence that environmental factors have any major influence on the development of arthritis but rather could be a consequence of the respiratory form of disease (Pfutzner, 1990).

In the human pathogen M. pneumoniae, extra pulmonary complications including arthritis have been reported mainly in children (Hakkarainen et al, 1992; Haier et al, 1999; Narita, 2010). The pathogenesis of these complications could be classified into three: inflammatory cytokines locally induced by surface lipoproteins in the *Mycoplasma*; an immune modulation

such as autoimmunity through cross-reaction between *mycoplasma*l components and human cells; and a vascular occlusion type in which vasculitis and/or thrombosis with or without systemic hypercoagulable state induced by M. pneumoniae (Narita, 2010). Arthritis in calves is temporary, therefore we speculate that its due to the first mechanism described above, that is, an inflammation due to temporary activation of cells in the joints.

Conclusion

Infection using a bronchoscope resulted in disease in both calves and adult cattle suggesting that intubation is a valid technique to infect and compare CBPP in cattle of different ages. We speculate that calves do not develop severe lung lesions because the lung epithelial cells of calves express low amounts of specific receptor molecules for Mmm, in contrast to homologous cells from older animals, including 7-month-old calves. This is supported by results from a previous experiment which reported that Mmm bound less efficiently to fetal calf lung epithelial cells than to adult lung epithelial cells (Aye et al, 2015). In contrast, adhesion of Mmm to epithelial cells in the joints may wane with age.

Competing interests

The authors declare that they have no competing interests.

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During the preparation of this article, Dr. Isabel Nkando unexpectedly passed away. Her warm spirit is being missed by all her colleagues

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PRELIMINARY STUDIES ON PATHOGENIC *LEPTOSPIRA* SPP. IN SLAUGHTERED PIGS IN ABEOKUTA, OGUN STATE, NIGERIA.

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Abstract

This study was undertaken to investigate the occurrence of pathogenic *Leptospira* spp. and the associated renal morphological changes in pigs slaughtered in slaughter slabs within Abeokuta metropolis, Nigeria. A total of 42 pigs' kidney samples were randomly collected for the study. The samples were examined using *Ellinghausen* McCullough Johnson Harris (EMJH) medium, microscopic agglutination test (MAT), Warthin Starry silver stain (WSss) and immunohistochemistry (IH). Thirty (71.43%) of the 42 kidneys showed visible macroscopic lesions. Interstitial nephritis and tubular nephrosis were the most prominent histopathological changes. *Leptospira* organisms were isolated from 35 (83.3%) of 42 kidneys. The occurrence of *Leptospira* species from 23 uncontaminated isolates using MAT was 95.7% (22/23). The reactant serovars and respective detection rate were *Icterohaemorrhagiae* (8/23, 34.8%), Pomona (4/23, 17.4%), *Gripptotyphosa* (3/23, 13%) Hardjo (3/23, 13%), Bratislava (2/23, 8.7%), *Canicola* (2/23, 8.7%), and 1 undetermined isolate (4.4%). *Leptospira* organisms were detected in 20 (87%) and 8 (34.8%) of the 23 renal tissues examined using WSss and IH respectively. Comparison of level of agreement among diagnostic methods [using kappa statistics (ki)] showed very low level of agreement between MAT and IH, and between WSss and IH. However, there was significant ($P<0.005$) measure of agreement between MAT and WSss. The significance of these *Leptospira serovars* in pig industry and their public health implications were discussed. This is the first investigative study on swine leptospirosis in Nigeria.

Keywords: immunohistochemistry, Leptospirosis, microscopic agglutination test, Pigs, renal pathology, Serovars.

ETUDES PRELIMINAIRES SUR LE LEPTOSPIRA SPP. PATHOGENE CHEZ LES PORCS ABATTUS A ABEOKUTA DANS L'ÉTAT D'OGUN AU NIGERIA

Resume

La présente étude a été réalisée dans le but d'étudier la présence du Leptospira spp pathogène et les changements morphologiques rénaux associés, chez les porcs abattus sur les postes d'abattage de la métropole d'Abeokuta au Nigeria. Au total, 42 échantillons de reins de porcs ont été prélevés de manière aléatoire aux fins de l'étude. Les échantillons ont été examinés en utilisant le milieu de culture Ellinghausen McCullough Johnson Harris (EMJH), le test d'agglutination microscopique (MAT), la coloration à l'argent Warthin Starry (WSss) et l'immunohistochimie (IH). Trente (71,43%) des 42 reins présentaient des lésions macroscopiques visibles. Les principaux changements histopathologiques observés étaient la néphrite interstitielle et la néphrose tubulaire. Des organismes de Leptospira ont été isolés dans 35 (83,3%) des 42 reins. La présence d'espèces de Leptospira provenant de 23 isolats non contaminés à l'aide de MAT

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était de 95,7% (22/23). Les sérovars réactifs et les taux de détection respectifs étaient Icterohaemorrhagiae (8/23, 34,8%), Pomona (4/23, 17,4%), Gripptotyphosa (3/23, 13%) Hardjo (3/23, 13%), Bratislava (2 / 23, 8,7%), Canicola (2/23, 8,7%) et 1 isolat indéterminé (4,4%). Des organismes de Leptospira ont été détectés dans 20 (87%) et 8 (34,8%) des 23 tissus rénaux examinés en utilisant respectivement WSss et IH. La comparaison du niveau de concordance entre les méthodes de diagnostic [en utilisant les statistiques Kappa (ki)] a montré un très faible niveau de concordance entre MAT et IH, et entre WSss et IH. Cependant, on a relevé un degré significatif (P <0,005) de concordance entre MAT et WSss. L'importance de ces sérovars de Leptospira dans l'industrie porcine et leurs implications sur la santé publique ont fait l'objet de discussion. C'est la première étude d'investigation réalisée sur la leptospirose porcine au Nigéria.

Mots-clés : immunohistochimie, leptospirose, test d'agglutination microscopique, porcs, pathologie rénale, sérovars.

Introduction

Leptospirosis and its attendant public health problems remains a neglected and clinically under-diagnosed condition in developing countries. Difficulty in recognizing the disease stems from its wide varieties of non-specific clinical manifestations and the problems encountered in making diagnosis or in isolating and identifying the exact serovars during infection (Sharma & Yadav 2008). Over the years, swine has been shown to be a maintenance host to serovars Pomona and Bratislava (Ellis 1999), but recently, the presence of other serovars such as *Icterohaemorrhagiae*, *Gripptotyphosa*, *Canicola* and *Hardjo* have been documented (Ellis 1999; Valencar *et al.,* 2012).

Clinical manifestations of leptospirosis in swine vary with infecting serovar. Infection with serovar Bratislava has been associated with low serologic response, rapid transmission from pig to pig, mild clinical signs resulting from transplacental infection and a prolong renal carrier state (Thiermann 1987). Reproductive-associated clinical signs of the disease are common findings in swine. These include infertility, late term abortion, stillbirth, mummified or macerated foetuses and increased neonatal mortality (Pritchard *et al.,* 1985). These manifestations of the disease have been the source of major losses to the swine industry (Bolin & Cassells 1990).

Confirmatory diagnosis of leptospirosis is laboratory based due to its non-specific clinical manifestations which mimic other febrile conditions (Vado-Solis *et al.,* 2002). Over the year, MAT and silver impregnation have been used regularly in the diagnoses of the disease in pigs (Faine 1965; Potts *et al.,* 1995). *Immunofluorescence* and *immunoperoxidase* have also been used to determine the presence and serovars of *Leptospira* organisms in the tissues of pigs (Ellis *et al.,* 1983; Skilbeck 1986, Scanziani *et al.,* 1989). More recently, polymerase chain reaction was employed in the diagnosis of leptospirosis in pigs (Azizi *et al.,* 2014). Apart from the work of Hunter *et al.,* (1987) and Potts *et al.,* (1995) in South Africa, for almost three decades, no significant study has been performed on swine *leptospirosis* in Africa, especially in terms of isolation and characterization of the prevalent serovars.

In Nigeria, only serological evidence of the disease in pigs in the northern part of the country has been documented (Ilozuec *et al.,* 2015). However, there is no single report on swine *leptospirosis* in major pig producing areas of southern part of Nigeria. The prevalence of the disease in ruminants (sheep, goat and cattle) was last reported by Agunloye, (2002) in the southwest Nigeria.

Until now, there were no detail documented information on swine leptospirosis in Abeokuta and the whole country. Thus, this preliminary study was designed to detect the presence of pathogenic *Leptospira* organisms in pigs slaughtered in Abeokuta, Ogun State, Nigeria, and its associated renal morphological changes using cultural isolation (CI), microscopic agglutination test (MAT), Warthin Starry silver stain (WSss) and immunohistochemistry (IH).

Materials and Methods

Study location

The study was carried out in Abeokuta, the capital city of Ogun State, southwest, Nigeria. The city of Abeokuta is located between latitude 70 15' N, longitude 30 35 ' E with relatively high average daytime temperature of above 28°C, and annual rainfall of 750 mm with average relative humidity of 74%. The city is about 157m above sea level, with an area of 16,762km^2 (Adekunle & Agbaje 2011). The State is one of the major pig producing states in Southwest Nigeria. Pigs were brought to the slaughter slabs within Abeokuta from the surrounding towns and villages by farmers.

Data Source and Sample Collection

Forty-two kidney samples, randomly selected from different pigs with unknown history, slaughtered at various slaughter houses within the city were selected for the present study. The samples were collected between June and July, 2012. Between 3 - 5 pigs are usually sampled per day in these various slaughter slabs except during festive periods in which more than the seen at least 5 to 7 pigs could be sampled per day. Variables such as age, sex and breed were documented. About 10-15g of kidney portions from each pigs was placed in individual sterile polythene bags and transported in ice-pack and taken to the Department of Veterinary Pathology, Federal University of Agriculture, Abeokuta for subsequent bacteriological and pathological analyses. The study was approved by the Ethical Committee on the use of experimental animals in the College of Veterinary Medicine, Federal University of Agriculture Abeokuta, Nigeria.

Preparation of EMJH Medium and Cultural Isolation

The isolation of *Leptospira* was made in *Ellinghausen*-MCcullough-Johnson-Harris (1965) broth medium (EMJH) (Difco-USA) with the addition of sterile 10% Rabbit serum and 5-fluorouracil (400 mg/L; Sigma®-USA), chloramphenicol (5 mg/L; Sigma®-USA), nalidixic acid (50 mg/L; Sigma®), neomycin (10 mg/L; Sigma®-USA) and vancomycin (10 mg/L; Across®-USA).

A small portion (1-2g) from each kidney sample was cut using sterile microtome blade, placed in Petri dish and 0.5 mL of Phosphate Buffer Saline (PBS) was added. The small portion of kidney sample was macerated in the PBS using sterile rat toothed forceps and allowed to remain in EMJH medium for 8-10 minutes. The macerated tissue was then inoculated into EMJH medium in bijou bottle. This was then incubated at room temperature (28-30°C) in the dark and subsequently examined under dark field microscope at intervals of 4 days to check for the growth of *Leptospira* organism for at least two months.

Leptospire Characterization

Microscopic agglutination test (MAT) was done according to the method of Obregon *et al.,* (2007), using rabbit monoclonal antibodies specific for six different serovars of *Leptospira* obtained from Prof. R. A. Hartskeerl (WHO/FAO/OIE and National Leptospirosis Reference Centre, KIT Biomedical Research, Amsterdam, The Netherlands). The specific monoclonal antibodies were labelled as follows: *Leptospira* interrogans *serovar Pomona*, *L. interrogans serovar Griptotyphosa*, *L. interrogans serovar Hardjo*, *L. interrogans serovar Bratislava*, *L. interrogans serovar Canicola*, and *L. interrogans serovar Icterohaemorrhagiae* (Table 1). Dilution of 1:100 of monoclonal antibodies were prepared in phosphate buffer saline (i.e. 1µl of monoclonal antibody + 100µl of PBS). 50µl of PBS were placed in each of well 2 to well 6 of a microtitre plate. Fifty microlitres of 1:100 monoclonal antibodies was then placed in well 1 and well 2 respectively. A two fold serial dilution was then made from well 2 to well 6. Fifty microlitres of the specimen (*Leptospire* culture of kidney sample) was added to all the wells from 1 to 6 in the microtitre plate. This was incubated at 37°C for 2 to 4 hours and view under the dark field microscopy. A reduction of at least 50% in the number of free leptospires in the test sample was considered positive with or without agglutination and was recorded as the respective titre (Senthil *et al.,* 2001).

Gross, Histopathology and Immunohistochemistry

The gross morphological changes were documented and their severity were graded as absent (-), mild (+), moderate (++) and marked (+++). Twenty-three uncontaminated kidney samples were fixed in 10% buffered formaldehyde and processed routinely for histopathology using haematoxylin and eosin stains (H&E), Section were also cut for Warthin-Starry silver impregnation. For histopathology, interstitial nephritis scores were assigned as positive (+) or negative (-) and its severity as follows; 1 = a focal area on section examined, 2 = a locally-extensive area on section examined, 3 = multiple foci or diffuse areas on section examined, while the presence of other lesions such as tubular degeneration and necrosis, tubular dilatation, hyaline cast were indicated as either positive (+) or negative (-). The presence or absence of the organism in tissues stained with WSss and immunohistochemistry were designated as either positive (+) or negative (-)

Immunohistochemically, serial 5 µm sections from each paraffin block were mounted on slides and allowed to dry overnight. The sections were deparaffinized in 4 changes of xylene for 3 minutes each, rehydrated in a graded levels of alcohol, and finally washed with deionised water. The antigens were unmasked from the deparaffinized sections with 0.01M sodium citrate buffer (pH 6.0) using microwave oven for 30 minutes at 650W. This was cooled to room temperature for 20 minutes and followed by 5 minutes of washing in 0.05M Phosphate Buffer Saline-Tween 20 (TPBS) (pH 7.6). All other steps were performed at room temperature. The sections were incubated in 3% hydrogen peroxide for 15minutes to quench endogenous peroxide. After a brief wash of TPBS, Histomark, (Biotin streptavidin-HRP System, Goat anti-Rabbit IgG (H+L) KLP, Gaitherburg U.S.A) detection system was used. Non-specific binding was done by bathing in normal goat serum for 10minutes. The tissues were then incubated with specific monoclonal antibody (1:800 dilutions in PBS) for 30 minutes. After a last wash in TPBS, the slides were incubated with streptavidin-biotin-horseradish peroxidase for 15 minutes. Slides were then rinsed with distilled water and incubated with 3-amino-9-ethylcarbazole-peroxidase chromogen for 10 minutes, rinsed with distilled water and counterstained with Mayer haematoxylin for 90 seconds, rinsed and mounted with glycerol for microscopic evaluation.

Statistical analysis

Data were analyzed using descriptive statistics. The prevalence rates among sex, age and breeds of pigs were expressed as percentage of the total number of animal sampled. Chi square test was used to evaluate association between rates of infection and sex, age and breeds. Kappa statistics was used to measure the level of agreement among WSss, MAT and IH used in this study.

Results

Bacteriological Studies

Culturally, *Leptospira* organisms were isolated from 35 (83.3%) of 42 kidneys from the slaughtered pigs. Fourteen (33.3%) out of the 42 kidney samples collected were males and 26 (61.9%) were females, while the sex of 2 (4.8%) could not be determined. Eleven (78.6%) out of the 14 samples from males were leptospire positive while 3 (21.4%) were negative. Twenty-four (92.3%) out of the 26 samples from females were leptospire positive while 2 (7.7%) were negative. The two samples in which the sex could not be determined were both leptospire negative (Table 2).

Thirty (88.2%) of the 34 samples from large white pigs were positive while the remaining 4 (11.8%) were negative. The 3 samples and one sample from local and the cross-breed were positive respectively. One (25%) out of the 4 samples from undetermined breed was positive while the other 3 (75%) were negative (Table 2).

Of all age groups, pigs between 6 months to 1 year had the highest isolation rate, with 16 (83.3%) of 18 kidney samples being positive, while animals of age group 3 to 6 months followed with 8 (72.7%) of 11 samples positive. Three (75%) of the 4 samples from

Table I: Reference Rabbit *leptospira* antisera used for characterization of *Leptospira* isolates from pigs.

Serogroup	Serovar	Strain
Canicola	*Canicola*	Hond Utrecht IV
Icterohaemorrhagiae	*Icterohaemorrhagiae*	RGA
Pomona	Pomona	Pomona
Grippotyphosa	Grippotyphosa	Moskva V
Sejroe	Hardjo type Prajitno	Hardjoprajitno
Australis	Bratislava	Jez Bratislava

Table 2: Detection of *Leptospira* organism according to sex, breed and age in 42 pigs in Abeokuta, Ogun State.

Risk factor	parameters	Total number	No. positive	Prevalence (%)	95%CI	p-value
City	Abeokuta	42	35	83.3	72-94	-
Sex	Female	26	24	92.3	82-100	0.21
	Male	14	11	78.6	58-100	
	Undetermined	2	-	-	-	
	Large white	34	30	88.2	77-99	
Breeds	Local breed	3	3	100	1-100	0.769
	Cross-breed	1	1	100	1-100	
	Undetermined	4	1	25	0-67	
Age	3 - 6mths	11	8	72.7	46 - 98	
	6 – 1yr	18	16	88..9	75 -100	0.51
	Above 1yr	4	3	75	33 -100	
	Undetermined	9	8	88.9	75 -100	

pigs above 1 year were positive. Moreover, 8 (88.9%) of 9 samples from undetermined age group were positive while 1 (11.1%) was negative (Table 2).

Macroscopic changes and Leptospira *serovars*

Out of the forty-two (n = 42) kidney samples randomly collected from the slaughter slabs, only 30 (71.4%) kidneys showed visible macroscopic changes (Table 3). These include; icterus (15, 35.7%), petechial haemorrhages (7, 16.7%), foci of cortical paleness ('white spots'), (8, 19.0%) and the remaining 12 (28.6%) kidneys were normal. Thirteen (86.7%) out of the 15 samples that showed icterus were *Leptospira* positive. Six (85.7%) of the 7 kidneys showing petechial haemorrhages were positive. Five (62.5%) of the 8 samples showing foci of cortical paleness were positive. In addition, 11 (91.7%) out of the 12 samples showing no macroscopic lesions on the kidney were *Leptospira* positive.

Of the 35 positive samples, 23 pure culture of *Leptospira* isolates, were characterized into different serovars using six monoclonal antibodies (Table 1). The typing of the isolates showed that *L. interrogans serovar Icterohaemorrhagiae* had the highest detection rate of 34.8% with 8 isolates and agglutination titre of 1:3200. This is followed by *L. interrogans serovar Pomona* with 4 (17.4%) isolates and agglutination titre of 1:800. There were 3 (13.0%) isolates each for *L. interrogans serovar Gripptotyphosa* and *L. interrogans serovar Hardjo* with agglutination titre of 1:3200 and 1:400 respectively. *Leptospira interrogans serovar Bratislava* and *L. interrogans serovar Canicola* had 2 (8.70%) isolates each and agglutination titre of 1:3200 and 1:800 respectively. However, the serovar of one (4.4%) of the 23 *Leptospira* isolates could not be determined (Table 3).

Table 3: Summary of gross lesions, cultural isolation, Microscopic agglutination test, Histopathology, Warthin Starry silver stain and Immunohistochemistry of 23 out of the 43 kidney samples of pigs.

S/N			Microscopic Agglutination Test		Histopathological changes				Severity of IN			
	GL	CL	+ / -(serovars)	Aggl. titre	TDN	IN	TD	HC	1	2	3	WSss
1	FCP	+	+(*L. icterohaemorrhagiae*)	1:3200	+	+	-	+	-	+	-	+
2	IC	+	+ (*L. gripptotyphosa*)	1:3200	+	+	+	+	+	-	-	+
3	FCP	+	+ (*L. hardjo*)	1:400	+	+	+	-	-	-	+	+
4	-	+	-	-	+	-	-	+	-	+	-	-
5	IC	+	+ (*L.icterohaemorrhagiae*)	1:3200	+	+	+	+	-	+	-	+
6	FCP	+	+ (*L. Bratislava*)	1:3200	+	+	+	-	+	-	-	+
7	IC	+	+ (*L.icterohaemorrhagiae*)	1:3200	+	+	+	+	-	+	-	+
8	-	+	+ (*L.hardjo*)	1:400	+	+	-	-	-	+	-	+
9	PH	+	+ (*L. Pomona*)	1:800	+	+	+	+	-	-	+	+
10	IC	+	+ (*L.icterohaemorrhagiae*)	1:3200	+	+	+	+	+	-	-	+
11	FCP	+	+ (*L. gripptotyphosa*)	1:3200	+	+	+	+	+	-	-	+
12	IC	+	+ (*L. Pomona*)	1:800	+	+	+	+	+	-	-	+
13	-	+	+ (*L.icterohaemorrhagiae*)	1:3200	+	+	+	+	+	-	-	+
14	IC	+	+ (*L. canicola*)	1:800	+	+	+	-	+	-	-	-
15	PH	+	+ (*L. Pomona*)	1:800	+	+	-	+	-	-	+	+
16	IC	+	+ (*L. gripptotyphosa*)	1:3200	+	+	+	+	-	+	-	+
17	PH	+	+ (*L.icterohaemorrhagiae*)	1:3200	+	+	+	+	+	-	-	+
18	IC	+	+ (*L.Bratislava*)	1:3200	+	+	+	-	-	+	-	+
19	IC	+	+ (*L. Pomona*)	1:800	+	+	+	+	+	-	-	+
20	-	+	+ (*L. canicola*)	1:800	+	+	-	-	-	+	-	+
21	-	+	+ (*L.icterohaemorrhagiae*)	1:3200	+	+	+	+	-	-	+	+
22	FCP	+	+ (*L. hardjo*)	1:400	+	-	+	+	-	-	+	+
23	IC	+	+ (*L.icterohaemorrhagiae*)	1:3200	+	+	+	+	-	-	+	-

Notes: GL= Gross lesions, CI= Cultural isolation, + = Positive, - = Negative/absent, Aggl. titre = Agglutination titre, TDN = Tubular degeneration and necrosis, IN = Interstitial nephritis, TD = Tubular dilatation, HC = Hyaline cast, FCP=foci of cortical paleness, IC= Icterus, PH=Petechial haemorrhages, MAT= Microscopic agglutination test, WSss = Warthin starry silver stain, IH = Immunohistochemistry, ND = Not determined..

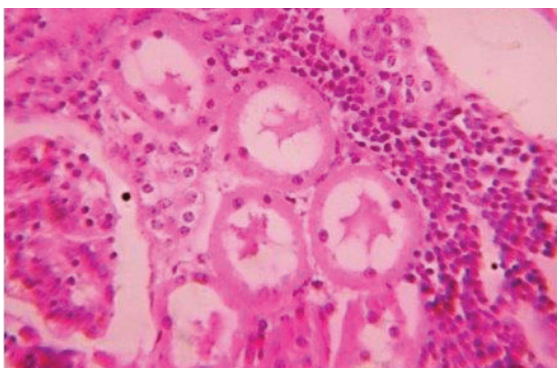

Figure 1: Kidney section showing moderate tubular degeneration and necrosis, tubular dilatation and moderate interstitial mononuclear cells infiltration with protein casts. Haematoxylin & Eosin, Bar = 50µm

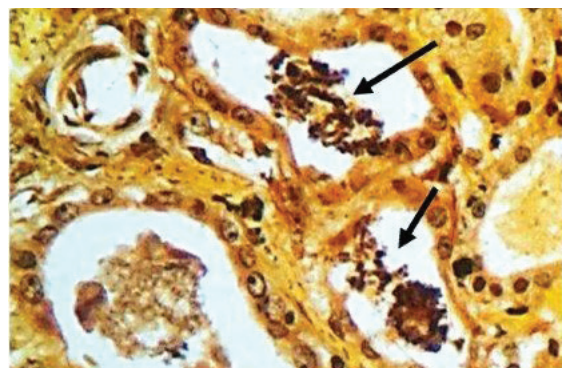

Figure 2: Kidney section demonstrating presence of *Leptospira* organisms in the lumen of the renal tubules. Warthin-Starry silver stain, Bar = 50µm.

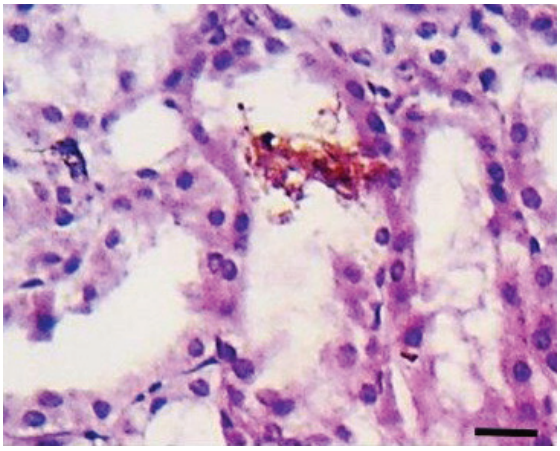

Figure 3: Kidney section showing immunohistochemical detection of *Leptospira icterohaemorrhagiae* antigens in the tubular lumen. Streptavidin-biotin immunoperoxidase, counterstained with haematoxylin. Bar = 50µm

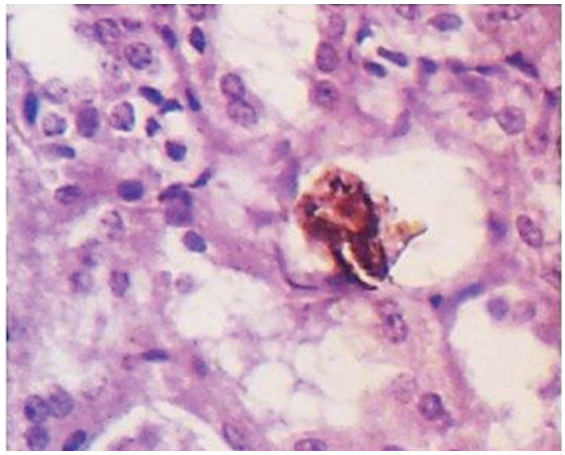

Figure 2: Kidney section demonstrating presence of *Leptospira* organisms in the lumen of the renal tubules. Warthin-Starry silver stain, Bar = 50µm.

Renal Histopathology

The histopathology of the 23 positive serotyped kidney samples revealed moderate to severe tubular degeneration and necrosis characterized by pyknosis, karyorrhexis and karyolysis in all the kidney sections examined. This is closely followed by mild to severe interstitial nephritis in 21 (91.3%) sections, characterized by interstitial, perivascular and periglomerular lymphoplasmacytic infiltration (Figure 1).The severity of the interstitial nephritis showed that 9 sections were mild, while 8 were moderate and 6 were marked or severe (Table 3). Tubular dilatation was observed in 18 sections (78.3%) while hyaline casts were present in 17 (73.9%). Other lesions such as renal congestion, interstitial oedema, interstitial fibrosis, tubular haemochromatosis, glomerulonephritis, tubular calcification, tubular dilatation, and tubular atrophy were also observed.

Out of the 23 kidney sections subjected to Warthin Starry silver stain 20 (87%) demonstrated either whole or dark granules of *Leptospira* organism within the tubular lumina (Figure 2), while 8 (57.14%) of the 23 sections immunohistochemically examined were positive to *L. interrogans serovar Icterohaemorrhagiae* (4, 28.6%) (Figure 3) and *L. interrogans serovar Pomona* (4, 28.6%) (Figure 4) (Table 3). The level of agreement between MAT and IH using kappa statistics was very low with kappa index of 0.047 and p-value of 0.45. The level of agreement between WSss and IH was also low with kappa index of 0.3 and p-value of 0.10. However, there was significant measure of agreement between MAT and WSss with kappa index of 0.47 and p-value of 0.008.

Discussion

This was the first investigative study on swine leptospirosis in Nigeria and in the continent of Africa using a combination of cultural isolation, microscopic agglutination test, Warthin Starry silver impregnation and immunohistochemistry. This study revealed high rate of *Leptospira* organisms (83.3%) in pigs slaughtered in Abeokuta metropolis. This rate of *Leptospira* detection was higher compared with the works of previous workers in swine. In the study of Azevedo *et al.,* (2008a) in Sau Paulo, Brazil, 14 (18.4%) of the 76 pigs examined were positive and in the studies of Delbem *et al.,* (2002), in the Northern Parana State, Brazil, only 24 (66.7%) of the 36 pigs examined were positive. However, the occurrence of swine leptospirosis was more than this study in the works of Scanziani *et al.,* (1989) in which 47 (60.3%) of the 74 tissues examined were

positive using immunoperoxidase procedure.

The significance of breeds of pigs in the epidemiology of leptospirosis is unknown. In this study, *Leptospira* organisms were detected in all the breeds of pig investigated. The higher detection of *Leptospira* in Large White than in other breeds could be due to overrepresentation since Large White was the most common breed of pigs slaughtered in the slaughter houses. However, the study revealed that all breeds of pigs are susceptible to *Leptospira* infection.

The importance of sex-related prevalence in the epidemiology of swine leptospirosis has not been elucidated in the literatures. In this study, although there was no significant difference between male and female, the higher number of female pigs might portend serious reproductive implications to pigs industry in southwest Nigeria (Azevedo *et al.,* 2008b). Lack of significance difference among age groups also suggests that all age groups of pigs are susceptible, but pigs between 6 months to 1year appeared to be more susceptible than other age groups. This is in agreement with the works of other workers who attributed risk factor of *Leptospira* infection to lower immunity in young pigs than the adult pigs (Thompson *et al.,* 2006).

The gross renal lesions, such as *petechial haemorrhages*, foci of cortical paleness called 'white spots' and icterus reported in this study were typical of renal changes in swine leptospirosis and were consistent with those previously documented in pigs and other animals (Scanziani *et al.,* 1989, Faine 1999). Previous studies have demonstrated an association between renal gross lesions and the presence of leptospire in the kidneys (Baker 1989, Hunter 1987). However, this study revealed that presence of renal gross lesions might not be an indication of *Leptospira* infection, since 6 kidney samples showed gross lesions but without *Leptospira* infection and 11 kidney samples were positive without renal gross lesions. This is in agreement with previous studies in which presence of leptospire in the kidneys were not associated with gross findings (Chappel *et al.,* 1992).

The histopathological changes of interstitial nephritis in porcine kidney in which *Leptospira* organism were isolated in this study were consistent with the work of Delbem *et al.,* (2002). Interstitial fibrosis might be due to response or sequelae of the renal tissue to previous inflammatory response possibly induced by pathogenic *Leptospira* organism. Other histopathological alterations like tubular dilatation, tubular vacuolar degeneration, renal casts and interstitial oedema were also consistent with the findings of other workers (Hunter *et al.,* 1987; Delbem *et al.,* 2002). The agreement between Warthin-Starry silver stain and immunohistochemical staining in this study as per the location of *Leptospira* on the epithelial cells or in the lumen of renal tubules was similar to that obtained by previous workers in kidneys of pigs (Scanziani *et al.,* 1989).

The predominant serovars in this study was *L. interrogans serovar Icterohaemorrhagiae*. This is in agreement with the report of Van til & Dohoo (1991), Delbem *et al.,* (2002) and Valencar *et al.,* (2012), who serologically detected higher frequency of antibodies to serovar *Icterohaemorrhagiae* in pigs than serovars Pomona and Bratislava which were traditionally associated with pigs. Previous studies have shown that (Cytenic *et al.,* 2003, Valencar *et al.,* 2012) rats were involved in the epidemiology of leptospirosis in pigs, especially those caused by serovar *Icterohaemorrhagiae*. More recently, rats were implicated in the epidemiology of African swine fever in Nigeria (Fasina *et al.,* 2012). Serovars Hardjo and *Gripptotyphosa* were the second most frequently observed serovars in this study. Cattle are known as the maintenance host for serovars Hardjo (Elzeh *et al.,*1987), while wild animals or rodents serve as reservoir hosts for serovars *Gripptotyphosa* (Valencar *et al.,* 2012). These two serovars have been associated with abortion and reproductive failures in pigs (Hathway *et al.,* 1983; Azevedo *et al.,* 2008a; Valencar *et al.,* 2012). In the study area, farmers are known for raising pigs and ruminants together on the same farm and pigs might have been an incidental host. Bratislava and *Canicola* were the third most recognized serovars. *Leptospira* belonging to Bratislava

serovar have been isolated from pigs in different parts of the world (Bolin & Cassells 1990; Ellis 1999) but the epidemiology of *Canicola* infection in swine is unknown. Over the years, dogs have been identified as the common maintenance host of serovar *Canicola*. Many farmers are known to have guard dogs in their farms in the study area. Therefore, it is possible that the presence of guard dogs on some of the farms where the pigs were raised were the primary source of infection and consequently made pigs an incidental host. Although *serovars Pomona, Hardjo, Bratislava*, *Canicola* and *Icterohaemorrhagiae*, have been culturally and serologically detected in pigs in South Africa (Hunter *et al.,*1987, Potts *et al.,* 1995) and serovars Bratislava serologically detected in the northern part of Nigeria (Ilozuec *et al.,* 2015), this study appears to be the first study on isolation and *serovar* identification of pathogenic *Leptospira* organisms in swine on the continent of Africa, especially in the major pigs producing area of Nigeria using combination of cultural isolation, Warthin Starry silver stain, microscopic agglutination test and immunohistochemistry to confirm the presence of leptospire in kidney tissues of pigs. Over the years, cultural isolation has been regarded as the most sensitive confirmatory method of diagnosis, but it is tedious, labour intensive, time wasting and unable to determine the exact serovars (Ellis 1999; Ilozuec *et al.,* 2015). In this study, the 23 pure *Leptospira* isolates and the respective renal tissues were subjected to MAT, WSss and IH to determine the presence of *Leptospira* organism and the infecting serovars within the renal tubules, as well as the level of agreement among the three diagnostic methods using kappa test. Although, there was no significant level of agreement between MAT and IH, and between WSss and IH, the 8 serovars confirmed by IH were also identified by MAT and WSss (Table 3). This shows that IH can be employed to confirmed cases of *Leptospira* infection in tissues and ultimately identifying the infecting serovars, using specific monoclonal antibodies. The remaining samples which were not confirmed by IH, but identified by MAT and WSss might be due to absence of *Leptospira* antigen in the small portion of the tissue examined immunohistochemically or insufficient *Leptospira* antigen to show positive signal in the sections as suggested by Ross et al, (1989). Moreover the significant ($P<0.05$) level of agreement between MAT and WSss shows that the combination of these two methods could be used to establish a confirmatory diagnosis when IH cannot be performed.

Conclusion

In conclusion, this study showed high occurrence or detection rate of *Leptospira* organism in pigs in the study area. It also emphasized the importance of combination of different diagnostic methods in the diagnosis and confirmation of the disease. This study has also demonstrated various serovars of *Leptospira* organism in pigs, with serovars *Icterohaemorrhagiae* being most frequently encountered. Thus, preventive measures such as effective hygiene and application of polyvalent vaccine (incorporating the identified serovars in this study) should be instituted on farms, considering the great economic losses which these serovars could inflict on pig industry in Nigeria. The potential public health hazards of swine leptospirosis to veterinary personnel, health workers, abattoir workers and the general public cannot also be underestimated. Therefore, preventive and sanitary measures should be put in place on pig farms and slaughter slabs to prevent human infection.

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HAEMATOLOGY AND SERUM BIOCHEMISTRY OF STARTER BROILER CHICKENS FED MALTED SORGHUM SPROUT (MSP) OR WHEAT-OFFAL BASED DIETS SUPPLEMENTED WITH YEAST CULTURE AND ENZYME.

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Abstract

An experiment was conducted to determine the haematology and serum biochemistry of starter broilers fed malted sorghum sprouts (MSP) or wheat offal (W/O)-based diets supplemented with yeast culture and enzyme. A total of two hundred and forty day- old, unsexed Marshal Broiler chickens used for the experiment were randomly allotted to 8 dietary treatment groups of 30 birds each. Each treatment group was replicated thrice with 10 birds per replicate. Data on selected blood indices of the birds were collected at the end of the course of the feeding trial that lasted for 4 weeks. The experiment was a 2X4 factorial consisting of two (2) test ingredients (MSP and wheat offal) at 4 inclusion levels of enzyme or yeast (0g kg^{-1}, +0.01gkg^{-1} yeast and Roxazyme G2$^{(G)}$ enzyme, + 0.01gkg^{-1} yeast, + Roxazyme G2$^{(G)}$ enzyme. At the end of the trial, the effect of MSP and wheat offal inclusion showed a significant effect ($P<0.05$) for uric acid and ALT.

However, uric acid, total protein, Albumin and Globulin were highest for wheat offal diet. Furthermore, the results show that values obtained for PCV, RBC, WBC, uric acid, total protein, albumin, globulin, creatinine, ALT & AST were significantly ($P<0.05$) affected by the enzyme, yeast and their combination. In conclusion, supplementation with yeast + enzyme improves total protein, albumin and globulin by broiler chickens. Therefore MSP diet should be supplemented with yeast+ enzyme or enzyme singly to improve Livestock ration for better utilisation and optimum performance.

Keywords: Haematological and Blood chemistry starter Broiler Malted Sorghum Sprout, wheat offal

HEMATOLOGIE ET BIOCHIMIE SERIQUE DE POUSSINS DE CHAIR SOUMIS AUX REGIMES A BASE DE GERMES DE SORGHO MALTE OU DE SONS DE BLE ADDITIONNES DE CULTURE DE LEVURE ET D'ENZYMES.

Resume

Une expérience a été réalisée dans le but de déterminer l'hématologie et la biochimie sérique des poussins de chair nourris aux germes de sorgho malté (MSP) ou aux sons de blé (E / H) additionnés de culture de levure et d'enzymes. Au total, deux cent quarante poussins de chair Marshal âgés d'un jour utilisés pour cette expérience ont été répartis de manière aléatoire à 8 groupes de traitement diététique de 30 oiseaux chacun. Chaque groupe de traitement a été répliqué trois fois, avec 10 oiseaux par répétition. Les données sur certains indices sanguins des oiseaux ont été recueillies à la fin de l'essai alimentaire qui a duré 4 semaines. L'expérience était un dispositif factoriel 2X4 constitué de deux (2) ingrédients à tester (MSP et sons de blé) à 4 niveaux d'inclusion d'enzymes ou de levure (0g kg-1, +0,01gkg-1 levure et Roxazyme G2 (G) enzyme, + 0,01gkg-1 de levure, + Roxazyme G2(G) enzyme. A la fin de l'essai alimentaire, l'effet du MSP et de l'inclusion de sons de blé a montré un effet significatif ($P <0,05$) pour l'acide urique et les ALT. Cependant, l'acide urique, la protéine totale, l'albumine et la globuline étaient plus élevés pour le régime aux sons de blé. En outre, les résultats montrent que les valeurs obtenues pour le PCV, le RBC, le WBC, l'acide

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urique, la protéine totale, l'albumine, la globuline, la créatinine, l'ALT et l'AST étaient significativement (P <0,05) affectées par l'enzyme, la levure et leur combinaison. En conclusion, la supplémentation en levure + enzyme améliore la protéine totale, l'albumine et la globuline chez les poulets de chair, donc le régime MSP doit être complété avec de la levure + enzyme ou l'enzyme uniquement afin d'améliorer la ration des poulets pour une meilleure utilisation et une performance optimale.

Mots-clés : hématologie, chimie sanguine, poussin de chair, germe de sorgho malté

Introduction

The poultry industry in Nigeria is expanding rapidly and broiler production is one of the important aspects of the industry. In addition, the success of poultry ventures depends upon the birds having good health, required nutrition and good management (Oluyemi and Roberts, 2000). The major factor militating against the prospect of this industry is the problem of inadequate supplies of conventional feed stuff at economic prices. Feed cost is the most expensive input in poultry production as it accounts for about 85 % of the total cost of production in poultry industry (Onifade and Babatunde, 1998). The search for alternative non- conventional feedstuff as partial or complete ingredient in poultry feed that is non- toxic and which maintain optimal immune response in poultry is therefore imperative.

Such non- conventional product is Malted Sorghum Sprout (MSP) a by-product of sorghum malting. MSP has good prospects as a livestock feed but its usefulness is limited by its tannin content and non-starch polysaccharides (Elkine *et al.,* 1995). In monogastric animals, one of the main adverse effects of tannins on nutrient utilization is the depression of pancreatic tyrosine and amylase activities (Ahmed *et al.,* 1991; Long staff and Mcitab, 1991). Results of many studies had suggested that tannin-containing sorghum is poorly utilized by poultry birds compared with non-tannin-containing sorghum or maize. Nutritionally, tannins interact with and precipitate protein during processing. The level of tannin in sorghum is enough to cause significant anti-nutritional effects, especially if the diet is inadequate in protein (Butter, 1990). MSP has been reported by Oduguwa *et al.,* (2001) to have a low nutritive value for monogastric animals and cannot be used as a main protein source. The low nutritive value may be due to the non- starch polysaccharide content and fibre as well as tannin in MSP. Therefore, there is a need for protein supplements in which yeast and the use of exogenous enzyme are considered appropriate to improve the nutritive value of cereal by- product. Friesen *et al.,* (1992) reported that the use of enzyme as feed additives in livestock feed has shown a lot of prospects as a way of improving the utilization of dietary nutrient and high fibre feedstuff by monogastric animals. It is also well documented that supplementation of diets with exogenous enzyme can reduce the adverse effects of some of these compounds especially those produced by carbohydrates and proteins (Bedford and Schulze, 1998). Enzyme had been reported to improve fibre digestion and reduces viscosity of digesta (Bedford *et al.,* 1991 and Atteh, 2001). Enzyme treatments have been used to improve performance of poultry chicks, increase weight gain, feed conversion efficiency, dry matter digestibility and nutrient digestibility and decrease jejunal content viscosity respectively (Sundu *et al.,* 2006, Oke *et al.,* 2014). Blood is an important index of physiological and pathological changes in an organism and has been used in assessing the body's ability to respond to nutritional challenges (Nworgu *et al.,* 2007; Aguihe *et al.,* 2012). *Heamatological* indices are essential in monitoring feed toxicity especially with feed constituents that affect the formation of blood (Aro and Akinmoyegun 2012). Bawala *et al.,* (2008) reported that nutritional studies should not be limited to performance, carcass quality and protein intake alone but the effect of feed materials on blood constituents is also an important evaluation to be investigated.

This study therefore seeks to investigate the haematological and serum biochemistry of starter broilers fed diet containing Malted Sorghum Sprout (MSP) or wheat offal (W/O) supplemented with enzyme and yeast.

Materials and Methods

Experimental site

The research work was carried out at the poultry unit of the Teaching and Research Farms, Federal University of Agriculture, Abeokuta, Ogun State Nigeria (Latitude7113' 49.46"N and Longitude3126'11.98"E).

Test ingredients (*Enzyme and yeast, Malted Sorghum Sprout and Wheatoffal*)

The commercial *enzyme* (*Roxazyme G®*) used in this study is a blend of multi-enzymes consisting of endo – 1, 4 – β – *xylanase* (EC 3.2.1.8), endo – 1, 3 (4) – β – *glucanase* (EC 3.2.1.6) and endo – 1, 4 – β – *glucanase* (EC 3.2.1.4) produced by Trichoderma reesei. Bakers' yeast was purchased commercially and used in this study. The Dried MSP used in this study was obtained from a commercial brewery industry located in Sango, Ogun State, and wheatoffal from the Federal University of Agriculture Abeokuta, Nigeria Agro-Allied Feedmill. Two hundred and forty (240) day –old Marshal Broiler chickens were obtained from a commercial hatchery and reared intensively on deep litter housing system. The birds were fed the experimental diets from day-old and were fed ad-libitum until mature weight at 4 weeks of age. The commercial enzyme used was added at the rate of 10g/100kg diet and yeast also at 10g/100kg diet. Eight experimental diets were formulated for starter (1-28days). Such that (150g/kg) MSP and wheat offal (WF) were supplemented with yeast (+Y), enzyme (+E) or a combination of yeast and enzyme (+Y+E) while unsupplemented diet (_Y_E) stands for control in broiler diet formulated.

Experimental birds, design and Dietary treatment

The experiment was a 2 by 4 factorial design made of 2 factors (MSP and WO and 4 levels (–Y-E, +Y, +E and +Y+E). The eight experimental diets were formulated such that (150g/kg) Malted Sorghum Sprout (MSP) and wheat offal (WO) were supplemented with yeast (+Y), enzyme (+E) or a combination of yeast and enzyme (+Y+E) while unsupplemented diet (_-Y_-E) stands for control in broiler starter diet formulated respectively. Two hundred and forty (240) day –old (Marshal Broiler chickens) obtained from a commercial farm were randomly allotted into 8 groups of 10 birds replicated 3 times and were allocated to 8 experimental diets. The feed were fed without restriction and managed on deep litter intensively.

Data collection and analysis

Serum chemistry

At 28 of the study blood samples were collected from 4 randomly selected broilers per treatment to determine the blood serum chemistry. Blood collection was done through brachial vein puncture (Frandson, 1986) using needles and syringes. Each blood sample was emptied into 2 sets of well labelled sample bottles; one containing ethylene diamine tetra-acetate (EDTA) as anti- coagulant while the other contained no anti-coagulant. The sample containing anti-coagulant was used to analysis for the haematological traits while the other sample that did not contain anti-coagulant was used to analyse the serum bio-chemical traits of the birds per treatment. Samples were analysed for haematological traits (packed Cell Volume (PCV), *Haemoglobin* (Hb), Red Blood Cell (RBC) and White Blood Cell (WBC) and biochemical traits (Blood glucose, the total serum protein, albumin and globulin were determined using bromocresol purple method (Varley *et al.,* 1980). Serum creatinine (Bousnes and Taussky, 1945) and serum uric acid concentration (Wootton, 1964) was determined according to standard procedures. Serum enzymes (alanine transaminase (ALT) and aspartate serum transaminase (AST) were analysed using the commercial kits (Qualigens India. Pvt. Ltd., Catalogue number 72201-04).

Statistical Analyses:

The proximate composition of the diet was determined by the AOAC (1995). The data generated were subjected to ANOVA in a completely randomized design using statistical Analysis System (SAS, 2001). Significant means at 5% level of probability were separated using Duncan's Multiple Range Test (Duncan, 1955)

Results

The proximate composition of Malted Sorghum Sprout and wheat offal are presented in table 2. MSP contained more ether extract, Ash, NDF, ADF and less crude protein, crude fibre than wheatoffal. The dry matter and NFE values recorded in this study were 842.3 g kg-1, 631.3g kg-1 respectively. The calcium, Phosphorus and HCN recorded of MSP were these 9.2 g kg-1, 11.1 g kg-1, 2.5mg/kg respectively. Effects of malted sorghum sprout (MSP) and wheat offal (W/O) with or without yeast and enzyme on blood parameters of broiler starter. The result showed that PCV, WBC, ALT were not significantly (P>0.05) affected by the treatments as presented in Table 3. RBC of birds on MSP diet supplemented with yeast and enzyme alone were significantly elevated. Those fed MSP diet without additives recorded the least (P<0.05) RBC value. The values of uric acid ranged between35.43 umol/L in birds fed MSP diet without additive to116.87 umol/L in birds fed MSP diet with enzyme supplemented. Birds fed MSP diets with enzyme alone, yeast alone and w/o diets with enzyme + yeast mixture recorded highest (P<0.05) uric acid value. The least value was recorded for birds fed MSP without additives. In addition, birds on MSP diet with yeast + enzyme had the highest (P<0.05) value (37.77g/L) for total protein and however recorded the least value (19.33g/l) for birds on MSP diet with enzyme supplementation.

Furthermore, the albumin value was significantly higher for the birds fed wheat offal diet without additive meanwhile, the least P<0.05) value was recorded for birds fed wheat offal diet with enzyme supplementation alone. Birds on MSP diet with yeast + enzyme had the highest (P<0.05) serum globulin value. However, least value was recorded for birds on MSP supplemented with enzyme, those without additive (-Y-E) and those on wheat offal diet with supplemented enzyme.

An increased value of creatinine was recorded for birds on wheat offal diet with enzyme supplementation while the least value was recorded for birds on wheat offal diet with yeast supplementation.

AST activities was significantly (P<0.05) affected by the dietary treatments. The AST was similar (P>0.05) for birds on wheat offal diet with yeast alone, wheat offal diet without additives and MSP diet with yeast + Enzyme but significantly higher than those of other treatments. The least AST activities were recorded for birds fed w/o with enzyme + yeast and enzyme alone.

Discussion

The proximate composition of malted sorghum sprouts and wheat offal revealed a dry matter values of 916.7g/kg^{-1} and 914.6g/kg for msp & w/o respectively. The NFE values recorded in this study were 560.7 g kg^{-1} and 538.6g kg^{-1} for MSP and W/O respectivelyThe NFE values recorded in this study were 560.7 g kg^{-1} and 538.6g kg^{-1} for MSP and W/O respectively. MSP contained more ether extract, Ash, NDF, ADF and less crude protein, crude fibre than wheatoffal. Crude protein (CP) value recorded for MSP here (227.3 g kg^{-1}) agrees with earlier reports of Aning et al. (1998), Fafiolu et al. (2006) and Akinola (2002), who reported CP values of 226 g kg^{-1} . The crude fibre (CF) of 36.9 g kg^{-1} reported here is at variance with the findings of Oduguwa *et al.,* (2001) who reported 83.0 g kg^{-1}. The low crude fibre of MSP here could be due to improper hardening or lignification of the rootlets and shoots has not taken place before growth termination. However, the high neutral detergent fibre (NDF) reported here is relatively higher compared with Oduguwa *et al.,* (2007) who reported a relatively high (NDF) (224 g/ kg) in MSP. This add credence to the fact that non-starch polysaccharides (NSP)

Table 1: The composition of basal experimental diet of the starter Broiler (g/kg)

Levels	**MSP**				**W/O**			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Ingredient	**-Y –E Control**	**+Y + E**	**+Y**	**+E**	**-Y –E Control**	**+Y + E**	**+Y**	**+E**
Maize	470	470	470	470	470	470	470	470
Wheat offal	-	-	-	-	150	150	150	150
Fish Meal	30	30	30	30	30	30	30	30
MSP	150	150	150	150	-	-	-	-
Soybean meal	160	160	160	160	160	160	160	160
Groundnut cake	146	146	146	146	146	146	146	146
Bone Meal	18	18	18	18	18	18	18	18
Oyster shell	15	15	15	15	15	15	15	15
Premix	3	3	3	3	3	3	3	3
Salt	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Lysine	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Methionine	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
TOTAL	1000	1000	1000	1000	1000	1000	1000	1000
Calculated Analysis								
ME (MJ/Kg)	11.32	11.32	11.32	11.32	11.51	11.51	11.51	11.51
CP (%)	24.11	24.11	24.11	24.11	23.02	23.02	23.02	23.04
EE (%)	3.56	3.54	3.54	3.54	4.06	4.06	4.06	4.06
CF (%)	2.95	2.95	2.95	2.95	3.78	3.78	3.78	3.78
Calcium (%)	1.18	1.18	1.18	1.18	1.18	1.18	1.18	1.18
Av. Phosphorus (%)	0.36	0.36	0.36	0.36	0.41	0.41	0.41	0.41
Lysine (%)	0.89	0.89	0.89	0.89	1.29	1.29	1.29	1.29
Methionine (%)	0.23	0.23	0.23	0.23	0.64	0.64	0.64	0.64
Determined Analysis								
CP (%)	22.73	23.73	23.62	23.28	22.96	23.91	23.48	23.52
EE (%)	3.86	3.68	3.57	3.68	3.59	3.54	3.61	3.52
CF (%)	3.69	3.60	3.48	3.52	3.97	3.51	3.53	3.51
NDF(%)	37.29	35.92	36.07	36.13	36.82	35.82	35.82	36.04
ADF (%)	17.12	15.29	15.31	15.62	16.53	15.06	15.73	15.42
NFE(%)	56.07	54.57	55.30	55.79	53.86	54.94	58.77	44.88
Ash (%)	5.32	5.98	6.06	5.89	5.26	5.73	6.16	6.08

†Vitamin/mineral premix provided the following per kg diet (grower diet): 200 g Ca; 77 g P; 710 mg F; 42 mg retinol; 1 mg cholecalciferol; 325 mg dl-tocopheryl acetate; 35 mg menadione; 45 mg thiamin; 125 mg riboflavin; 75 mg pyridoxine; 300 µg cyanocobalamin; 875 mg niacin; 19 mg folic acid; 300 mg pantothenic acid; 7.5 g choline; 31 g methionine; 2500 mg Mn; 1500 mg Zn; 1250 mg Fe; 250 mg Cu; 15 mg I; 8.2 mg Se.

Table 2: Chemical compositions of test ingredient

Test ingredients	MSP	W/O
Component	g kg-1	g kg-1
Dry matter	916.7	914.6
Crude protein	227.3	229.6
Crude fibre	36.9	39.7
Ash content	53.2	52.6
Ether extract	38.6	35.9
Nitrogen free extract	631.3	538.6
Ca	9.2	8.05
P	11.1	10.2
HCN (mg/kg)	2.5	
NDF	372.1	368.2
ADF	171.2	165.3

limiting the utilization of this by-product. Also the high NDF, ADF and crude fibre contained in MSP confirm its content of non-starch polysaccharides. Report had shown that MSP has good prospects as livestock feed but its usefulness is limited by its tannin content and non-starch polysaccharides Elkine et al. (1995). In addition, the ether extract recorded agrees with earlier reports of Akinola (2002) who recorded 39.8 g kg^{-1} for alkaline-treated MSP and Aning *et al.,* (1998) who reported 33.0 g kg^{-1}. The ash content of 53.2 g kg^{-1} obtained here agreed with the findings of Fafiolu *et al.,* (2016), who reported 63.0gkg^{-1} Akinola (2002), who reported 70.0, 60.0 and 95.0 g kg^{-1} for untreated fermented and alkaline treated for ash content in MSP.

The calcium (Ca) and Phosphorus (P) contents of msp in this result is at variance with Oduguwa *et al.,* (2007) reported the calcium (Ca) content of MSP was 1.78 g kg^{-1} and 3.5g kg^{-1} respectively.

The higher value of uric acid in birds fed wheat offal and MSP suggests that protein in these diets were poorly utilized by the birds. The reduction in uric-acid indicates a slight improvement in protein utilization. However, the increased uric acid with inclusion of yeast is an indication that the birds did not utilize the protein properly which accounted for the poor performance of the final live weight of the birds. Kumpta and Harper (1961) and Eggum (1970) explained that an amino acid imbalance will result in an increase in blood urea concentrations. Urea is known to be a function of protein quality as high level is an indication of low protein quality. Ranjhan (2001) explained that in a diet, the amino acid present will be deaminated and hence result in an increase in excretion of urea. This agreed with earlier work of (Oke *et al.,* 2014; Fafiolu *et al.,* 2016) who reported increased uric acid when turkeys and pullet chicks diets were supplemented with MSP.

The activity of serum glutamate pyruvate transaminase enzyme was higher in birds fed MSP based diets. The activity of this enzyme is normally very low except in cases when the nutritional plane is very low or the presence of a toxic factor which may affect the liver. The higher activity of this enzyme recorded for birds fed MSP diet add credence to the fact that MSP contain some toxic factors. It has been reported to contain tannin (Aning *et al.,*1998; Oduguwa *et al.,* 2001) and hydro cyanide (Ikediobi, 1989).

The Red blood cell, total protein, albumin, globulin & AST were significantly ($P<0.05$) influenced by additives at starter phase. The values recorded for AST in this research was considerably higher in comparism

Table 3: Effect of MSP and W/O with yeast (+y) and enzyme (+e) on blood parameters of starter broiler chickens.

	Starter								
	MSP				W/O				
Measurements	**-Y-E**	**+Y+E**	**+Y**	**+E**	**-Y-E**	**+Y+E**	**+Y**	**+E**	**SEM**
HAEMATOLOGICAL PARAMETERS									
Packed cell volume (%)	21.00	26.00	25.33	24.33	23.00	26.33	23.33	24.00	0.51
Red blood cell (106/l)	5.27^{f}	5.47^{ef}	6.97^{a}	6.73^{a}	5.97^{c}	6.37^{b}	5.53^{de}	5.76^{cd}	0.14
White blood cell (109/l)	8.10	8.50	10.03	9.47	8.97	9.23	9.60	9.50	0.16
BLOOD CHEMISTRY									
Uric acid (umo/l)	35.43^{f}	56.37^{e}	113.53^{a}	116.87^{a}	106.07^{b}	114.00^{a}	83.83^{c}	67.57^{d}	6.16
Total protein (g/l)	20.57^{f}	37.77^{a}	25.31^{d}	19.33^{g}	33.00^{b}	31.47^{c}	23.06^{e}	18.08^{h}	1.42
Albumin (g/l)	10.60^{d}	12.63^{c}	13.43^{b}	9.70^{e}	15.10^{a}	12.33^{c}	10.44^{d}	9.03^{f}	0.42
Globulin (g/l)	9.97^{e}	24.30^{a}	11.97^{d}	9.67^{e}	17.90^{c}	19.09^{b}	12.75^{d}	9.08^{e}	1.11
Creatinine (mmol/l)	117.10^{e}	121.80^{d}	100.20^{g}	127.30^{c}	109.63^{f}	129.90^{b}	81.97^{h}	139.27^{a}	3.59
ALT (u/L)	12.67	20.00	13.33	18.67	7.33	16.67	8.67	10.67	0.95
AST (u/L)	18.67^{b}	22.00^{a}	18.00^{b}	18.67^{b}	22.33^{a}	12.67^{c}	22.00^{a}	13.33^{c}	0.82

abc Means on the same row having different superscripts are significantly different ($p<0.05$)

with Fafiolu (2003). This could be as a result of poor utilization of nutrient in the feed. Fafiolu *et al.,* (2006) reported that an increase in activities of the transaminase indicates poor utilization of nutrients in the feed.

Furthermore, the positive effect of additives supplementation on RBC recorded implied that birds fed diets supplemented with increased mixtures of enzyme + yeast had high oxygen carrying capacity (Brij *et al.,* 1977). It is an indication that the nutritional profile of the diet was more enriched when supplemented with high level of additives. Nutrition was reported to influence the *haemoglobin* level of the blood (Udo, 1987). Pellet and Young (1980) confirmed that *haemoglobin* levels are positively correlated with protein quality and level in the diets. The higher creatinine of birds fed MSP diet with yeast + enzyme and birds on wheat offal diets supplemented with yeast or enzyme is an indication that the birds were unable to incorporate the protein in the feed into their tissues. (Eggum, 1970 and Adewusi 1993 reported that High level of creatinine in the blood is an indication of impair protein utilisation. In conclusion, Malted Sorghum Sprouts cannot replace wheat offal without appropriate supplementation with additives or their combinations for optimum performance.

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HEMATOLOGICAL AND MALE HORMONAL PROFILE OF TWO CATTLE BREEDS REARED AT THE FEDERAL UNIVERSITY OF AGRICULTURE, ABEOKUTA.

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Abstract

Baseline data on the hematology of White Fulani and Muturu breeds of Cattle have been reported. However, very few documented studies have been carried out with regards to the reproductive hormonal profile of these breeds. Satisfactory reproductive performance is important to effective management and production as a whole in cattle production. Provision of data representing the male reproductive profile is imperative in view of the increasing importance of assisted reproduction and genetic selection in livestock farming. In the present study, the total erythrocyte values were similar in the male and female animals of both breeds. PCV and reticulocyte count in males were significantly higher than in females of the White Fulani breed while erythrocyte and leucocyte parameters obtained for the Muturu cattle were also similar for the males and females, except the reticulocyte values which were significantly higher in the females. Testosterone concentrations obtained for the Muturu bulls were similar to those earlier reported for Brown Swiss bulls (Thun *et al.,* 1980) while values reported for the White Fulani breed are also similar to those for Nguni bulls (Kay, 1984). Average plasma FSH concentrations of the White Fulani breed were higher than those for the Muturu breed in this study. However, the mean plasma LH concentrations were higher in the Muturu breed. These data provide a baseline from which to compare *hematological* and male reproductive hormonal values in White Fulani and Muturu cattle and will be especially valuable in future in designing therapeutic protocols for the managements of abnormalities in these breeds of cattle.

Key words: *Hematology*, reproductive, hormones and cattle

PROFIL HÉMATOLOGIQUE ET HORMONAL MALE DE DEUX RACES DE BOVINS ÉLEVÉES À L'UNIVERSITÉ FÉDÉRALE D'AGRICULTURE D'ABEOKUTA

Resume

Les données de base sur l'hématologie des races bovines Fulani blanc et Muturu ont été mises à disposition. Cependant, très peu d'études documentées ont été réalisées en ce qui concerne le profil hormonal reproductif de ces races. Une performance reproductive satisfaisante est importante pour une gestion et une production efficaces dans l'ensemble de l'élevage bovin. La mise à disposition de données sur le profil reproductif mâle est impérative compte tenu de l'importance croissante de la reproduction assistée et de la sélection génétique dans l'élevage. Dans la présente étude, les valeurs globales d'érythrocytes étaient similaires chez les animaux mâles et femelles des deux races. Les PCV et numération de réticulocytes chez les mâles étaient significativement plus élevés par rapport à ceux des femelles de la race Fulani blanc, tandis que les paramètres des érythrocytes et des leucocytes obtenus pour les bovins Muturu étaient similaires pour les mâles et les femelles, à l'exception des réticulocytes qui étaient significativement plus élevés chez les femelles. Les taux de testostérone obtenus pour les taureaux Muturu étaient similaires à ceux précédemment observés chez les taureaux Swisse brune (Thun et al., 1980) tandis que les valeurs rapportées pour la race Fulani blanc sont également similaires à celles des taureaux Nguni (Kay, 1984). Les taux plasmatiques moyens de FSH de la race Fulani blanc étaient plus élevés que ceux de la race

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Muturu dans cette étude. Cependant, les taux plasmatiques moyens de la LH étaient plus élevés chez la race Muturu. Ces données donnent une base de comparaison des valeurs hormonales hématologiques et reproductrices mâles chez les bovins des races Fulani blanc et Muturu, et seront particulièrement utiles à l'avenir dans la conception de protocoles thérapeutiques pour la prise en charge des anomalies dans ces races bovines.

Mots-clés : hématologie, reproductif, hormones et bovins

Introduction

Blood parameters are important indicators of health and disease status in animals and have become indispensable tool in the prevention, diagnosis, treatment protocol and/or the prognosis of many disease conditions in animals. Blood is an important index of physiological and pathological changes in an organism (Mitruka and Rawnshey, 1977). Haematological values are widely used to determine systematic relationship and physiological adaptation including the assessment of general health condition of animal (Kamal *et al.,* 2007)

Haematological parameters of many breeds of cattle have been evaluated (Mbanasor *et al.,* 2003, Olayemi *et al.,* 2007). Many of these parameters are known to be affected by such factors as age, sex, breed, season and physiological status of the animal and environmental conditions (Kausslish and Arora, 1977; Schalm *et al.,* 1975; Ewuola *et al.,* 2004).

Baseline data on the hematology of White Fulani and Muturu cattle breeds have been reported (Oduye and Okunaiya, 1971; Saror and Coles, 1973; Olayemi, Olawale and Fajimi, 2001; Olayemi, Nwandu and Aiyedun, 2007) which needs regular review.

Hormones are chemical transmitter substances produced by cells of the body and transported by the blood and other means to the cells and organs which carry specific receptors for them and on which they have a specific regulatory effect (Greco and Stabenfeldt, 2007). Hormones act as chemical messengers to body organs, stimulating certain life processes and retarding others. Growth, reproduction, control of metabolic processes, sexual attributes and behavior are dependent on hormones. (Kamal *et al.,* 2007)

However, very few documented studies have been carried out with regards to the reproductive hormonal profile of these breeds. This study therefore, attempts to provide some baseline data to aid the interpretation of male reproductive hormonal and hematological levels in both breeds.

Materials and Methods

Twenty (20) animals from the Directorate of the University Farm, Federal University of Agriculture, Abeokuta were sampled: 10 male animals (5 White Fulani and 5 Muturu) and 10 female animals (5 each of the White Fulani and Muturu breeds).

Experimental protocols

Determination of the Haematological Parameters.

Five (5) ml of blood samples via jugular venipuncture were taken from all 20 animals into heparinized tubes for haematology.

Packed cell volume (PCV) was determined by filling a plain capillary tube with blood to about three-quarter length of the tube and the vacant end of the tube was sealed using plasticine. The sealed tubes were then centrifuged at a revolution of 3,000/ minute for five minutes. Each tube was placed in microhaematocrit reader and the packed cell volume value was obtained and expressed in percentages.

The *haemoglobin* (HB) concentration was determined spectrophotometrically according to the method of Franco (1984) as described in Cypress diagnosis kit. The Red blood cell (RBC) values were determined by use of the haemocytometer. Blood was diluted in ratio1:200 with red blood diluting fluid using the red blood cell pipette. The dilution

was mixed and left for 3minutes after which the counting chamber of haemocytometer was charged and the red blood cell was counted using the ×40 objective of a microscope.

The total number of cells counted was multiplied by 10,000 and expressed in cubic millimeter (mm3) or litre. Total white blood cell (WBC) was determined using the white blood cell pipette of the haemocytometer, blood was diluted in ratio 1:20 with white blood cell diluting fluid.

The solution was gently mixed together and the counting chamber of the haemocytometer was charged with the solution and the total white blood cell was counted using the ×10 objectiveof the microscope. The total number of cell counted was multiplied by 50 and expressed in mm3 or litre. White blood cell differentials were carried out by making a thin film of blood on a clean grease-free slide using a smooth-edged spreader. The blood film was fixed with absolute methyl-alcohol for 3 – 5mins and allowed to dry. The film was stained with Giemsa stain and 100 white blood cells were differentiated using the oil immersion objective of microscope.

Hormonal Assay

Three (3) ml of blood samples from the male animals for the hormonal assay were collected by venipuncture from the jugular vein periodically (every 20mins for 1 hour). The blood samples were centrifuged (1,700 ×g, 15 min, 5°C) and the plasma samples obtained were stored at –20 C until the assay. The levels of testosterone, FSH and LH in plasma were measured by enzyme-linked immunosorbent assay (ELISA) as described by Isobe and Nakao (2004) and Optical density was measured using a microplate reader (Model 550; Bio-Rad Laboratories, Hercules, CA, USA) at 492 nm.

Statistical analysis

The GraphPad Prism 6 ® statistical package was used for the analysis of all data obtained during the course of this study making use of Student's t-test and Analysis of Variance (ANOVA) test in comparison of variables as well as determination of significance in difference ($P < 0.05$ was considered significant).

Results & Discussions

In the present study, the total erythrocyte values were similar in the male and female animals of both breeds. PCV and reticulocyte count in males were significantly higher than in females of the White Fulani breed while erythrocyte and leucocyte parameters obtained for the Muturu cattle were also similar for the males and females, except the reticulocyte values which were significantly higher in the females.

PCV and RBC values obtained for the White Fulani in this study are similar to previous records (Olayemi *et al.,* 2006) and PCV of Muturu (Olusanya *et al.,*1979). (Table 1) PCV value obtained from this study for Muturu cattle is similar to previous records (Olusanya *et al.,* 1979). Total white blood cell count and neutrophil count were significantly higher in the Muturu. (Table 1). Total erythrocyte values were similar in both sexes of the breeds agreeing with previous findings in Keteku (Awolaja *et al.,* 1997), White Fulani catlle, (Olayemi et. al., 2004) and Kuri cattle (Olayemi *et al.,* 2006). (Table 2). PCV and reticulocyte count in White Fulani males were significantly higher and reticulocyte values were significantly higher in female Muturu (Table 2). Olayemi and his colleagues reported erythrocyte values of White Fulani breeds as 9.50±3.09´1012/L (Olayemi *et al.,* 2007), lower than ours. (Table 1)

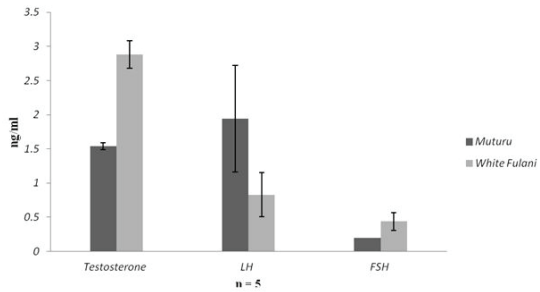

Figure 1: Showing hormone values (Mean+S.E.M) of the Muturu and White Fulani breeds of cattle

Table 1: Erythrocyte and leucocyte values (mean+S.E.M) of the White Fulani and Muturu breeds of cattle

PARAMETERS	WHITE FULANI	MUTURU
PCV (%)	31.70±0.75	33.80±1.31
RBC (´10^{12}/L)	7.14±0.38	7.10±0.46
Reticulocytes	2.50±0.49	2.45±0.51
WBC (´10^{9}/L)	7.43±0.29	9.09±0.57*
Neutrophils (´10^{9}/L)	2.37±0.09	3.07±0.21*
Lymphocytes (´10^{9}/L)	4.99±0.26	5.94±0.46
Eosinophils (´10^{9}/L)	0.01±0.01	0.01±0.01
Basophils (´10^{9}/L)	0.03±0.02	0.03±0.02
Monocytes (´10^{9}/L)	0.03±0.02	0.03±0.02

**P < 0.05*

Table 2: Erythrocyte and leucocyte values (mean+S.E.M) of the White Fulani breed of cattle as influenced by sex

PARAMETERS	MALES	FEMALES
PCV (%)	33.2 ±0.8	30.20±0.86*
RBC (´10^{12}/L)	7.42±0.65	6.86±0.44
Reticulocytes	3.70±0.46	1.30±0.41*
WBC (´10^{9}/L)	7.80±0.3688	7.06±0.41
Neutrophils (´10^{9}/L)	2.35±0.12	2.40±0.15
Lymphocytes (´10^{9}/L)	5.37±0.33	4.61±0.36
Eosinophils (´10^{9}/L)	0.03±0.17	0.00±0.00
Basophils (´10^{9}/L)	O.O3±0.01	0.03±0.03
Monocytes (´10^{9}/L)	0.04±0.03	0.14±0.14

**P < 0.05*

Table 3: Erythrocyte and leucocyte values (mean+S.E.M) of the Muturu breed of cattle as influenced by sex

PARAMETERS	MALE	FEMALE
PCV (%)	33.20±1.21	32.30±1.01
RBC (´10^{12}/L)	7.25±0.50	6.99±0.31
Reticulocytes	2.45±0.49	2.50±0.51*
WBC (´10^{9}/L)	8.79±0.61	7.73±0.36
Neutrophils (´10^{9}/L)	2.68±0.22	2.77±0.18
Lymphocytes (´10^{9}/L)	6.01±0.42	4.93±0.30
Eosinophils (´10^{9}/L)	0.02±0.01	0.00±0.00
Basophils (´10^{9}/L)	0.04±0.02	0.03±0.02
Monocytes (´10^{9}/L)	0.05±0.02	0.01±0.01

**P < 0.05*

Testosterone concentrations obtained for the Muturu cattle were similar to those earlier reported for Brown Swiss bulls (Thun *et al.,* 1981) while values reported for the White Fulani breed are also similar to those for Nguni bulls (Kay, 1985) (Table 4). Mean plasma testosterone and FSH concentrations of the White Fulani breed were higher than those for the Muturu breed in this study (Table 4). However, the mean plasma LH concentrations were higher in the Muturu breed. The plasma LH concentrations in the study for both breeds are higher than those recorded in other studies (D'Occhio *et al.,*1996) (Table 4).

Conclusion

These data obtained can be useful as baseline from which to compare hematological and male reproductive hormonal values in White Fulani and Muturu breeds of cattle and these values will be valuable for future studies on these breeds of cattle. It could also represent a tool in the health assessments of the two breeds.

Further works can be carried out on these breeds as well as other breeds and species, using larger sample sizes, to investigate deeper the reasons of differences in values recorded.

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ASSOCIATION OF SMALLHOLDER DAIRY FARMERS MANAGEMENT AND MILKING PRACTICES WITH BACTERIAL QUALITY OF MILK IN MBEYA, TANZANIA

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Abstract

The study determine the association between some of managements and milking practices with bacterial counts in dairy herd among small holder dairy farmers of Mbeya and Mbozi districts of Mbeya, Tanzania. A cross-sectional study was conducted with the aim of assessing housing structures, condition and management, milking procedure and milk handling in the study area. A total of 192 raw milk samples were collected from farmers herds with at least one lactating dairy cow. Samples were tested for total bacteria count (TBC), total coliform counts (TCC) and total staphylococcus counts (TSC) using standard procedures. All respondents practiced hand milking and 96.9% of the respondents washed hands before milking. About 57.8% of the respondents used same towel to dry the udder of all milking cows in the herd per milking time. Furthermore, 42.2% of the respondents used individual towel for drying udder and teat. None of the respondents used pre milking, post milking dipping or dry cow therapy. Milk from cows kept in barns made from concrete floor had ($p<0.001$) lower TBC and TSC. Frequency of cleaning of dairy barn ($p<0.05$) influenced the TBC, TCC and TSC. Lack of fore milking ($p<0.05$) associated with higher TBC. Furthermore, water source ($p<0.05$) influenced TBC and TCC. Milking practices which includes washing of hands, udder and teat, dry of teats using individual towel per cow and followed by fore milking yielded ($p<0.05$) lower bacterial count than other practices. Similarly, cow barns whose floors were made from concrete and cleaned twice or more daily had clean cow which produce milk with lower bacterial counts than cows kept on floors made from timber and soil. The results reveal the need for training farmers in good agricultural practices. This would contribute to achieving better quality milk, and ensure the sustainability of the sector in the study area.

Key words: Cow barn, fore milking, post milking, Total bacteria count, Total coliform counts, Total *staphylococcus* counts

ASSOCIATION ENTRE LES PRATIQUES DE GESTION ET DE TRAITE DES PETITS PRODUCTEURS LAITIERS ET LA QUALITE DU LAIT SUR LE PLAN BACTERIOLOGIQUE A MBEYA EN TANZANIE

Resume

L'étude détermine l'association entre certaines pratiques de gestion et de traite et les numérations bactériennes dans le troupeau laitier des petits producteurs laitiers des districts de Mbeya et de Mbozi à Mbeya en Tanzanie. Une étude transversale a été menée dans le but d'évaluer les structures, l'état et la gestion des logements, la procédure de traite et la manipulation du lait dans la zone d'étude. Au total, 192 échantillons de lait cru ont été recueillis auprès de troupeaux d'éleveurs ayant au moins une vache laitière en lactation. Les échantillons ont été examinés pour déterminer la numération bactérienne totale (TBC), la numération totale de coliformes (TCC) et la numération totale de staphylocoques (TSC) en utilisant des procédures standard. Tous les répondants pratiquaient la traite manuelle et 96,9% des répondants se

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lavaient les mains avant la traite. Près de 57,8% des répondants utilisaient la même serviette pour sécher la mamelle de toutes les vaches laitières du troupeau par moment de traite. De plus, 42,2% des répondants utilisaient une serviette individuelle pour sécher la mamelle et le trayon. Aucun des répondants n'utilisait la thérapie de prétraite, de trempage post-traite ou de tarissement. Le lait des vaches gardées dans des étables avec plancher en béton avait ($p < 0,001$) une TBC et une TSC plus faibles. La fréquence du nettoyage de l'étable des vaches laitières ($p < 0,05$) a influé sur la TBC, la TCC et la TSC. L'absence de prétraite ($p < 0,05$) était associée à une TBC plus élevée. De plus, la source d'eau ($p < 0,05$) a influencé la TBC et la TCC. Les pratiques de traite comprenant le lavage des mains, des mamelles et des trayons, le séchage des trayons à l'aide d'une serviette individuelle par vache et suivi de prétraite ont donné ($p < 0,05$) une numération bactérienne plus faible par rapport aux autres pratiques. De même, les vaches logées dans les étables dont les planchers étaient en béton et nettoyées deux fois ou plus par jour étaient propres et produisaient du lait dont la numération bactérienne était inférieure à celle des vaches gardées sur des planchers de bois et de terre. Les résultats révèlent la nécessité de former les éleveurs aux bonnes pratiques d'élevage. Ceci contribuera à obtenir du lait de meilleure qualité et à assurer la durabilité du secteur dans la zone d'étude.

Mots-clés : étable à vaches, prétraite, post-traite, numération bactérienne totale, numération totale de coliformes, numération totale de staphylocoques

Introduction

Bacterial contamination in farmer's bulked milk reduces the shelf life of milk and the quality of dairy products. Hygienic practices during milking are aimed at keeping bulked milk bacterial counts at acceptable levels. Sources of bacterial contamination in bulked milk during milking are the surfaces of milking utensil, the external surface of teats, udder, and mastitis pathogens from within the udder (Murphy and Boor, 2000).

Milk quality starts on the farms where production takes place and it is the place where primary level of quality is directly linked to the activities undertaken during the production process. Levels of bacterial contamination in raw milk are influenced by hygiene of dairy cows, health, the environment where dairy cows are housed and milked, methods of udder preparation and milking technique, speed of milk cooling and milk storage time (Fatine *et al.,* 2012). In order to keep bacteria count at acceptable levels, hygienic practices during milking time are essential (Kurwijila, 2006). Because bacterial contamination in milk not only reduces its nutritional quality but also consumption of such milk threatens health of the consumers (Mdegela *et al.,* 2009). In view of that Total Bacteria, Total Coliform and Total Staphylococcus count may be used as parameters for assessing hygiene in milk as they indicate the conditions in which the raw milk is produced, stored and subsequent handling prior to product manufacturing processes. Omore *et al.,* (2004) reported that the risk of bacterial contamination originating at farm level increases with bulking and number of agents handling milk before it reaches the final consumer. The way in which milk is produced at the farm may have negative impact on its quality if not produced in clean environment and handled hygienically. Several workers (Vairamuthu *et al.,* 2010; Duguma and Jassens, 2015; Najand and Rezaii, 2015) pointed out the risk factors for milk contamination during production, but there is limited work so far undertaken regarding the association of various management and milk handling practices to bacteriological quality of raw milk in Mbeya region, Tanzania. Also there is limited data on hygienic practices conducted throughout the dairy production in the study area and standard milking protocols are not followed and therefore, this study aimed to investigate the association between some of farm management, milking practices and raw milk bacterial quality in Mbeya region.

Materials and Methods

Study area

The study was conducted in two districts namely Mbozi and Mbeya rural district

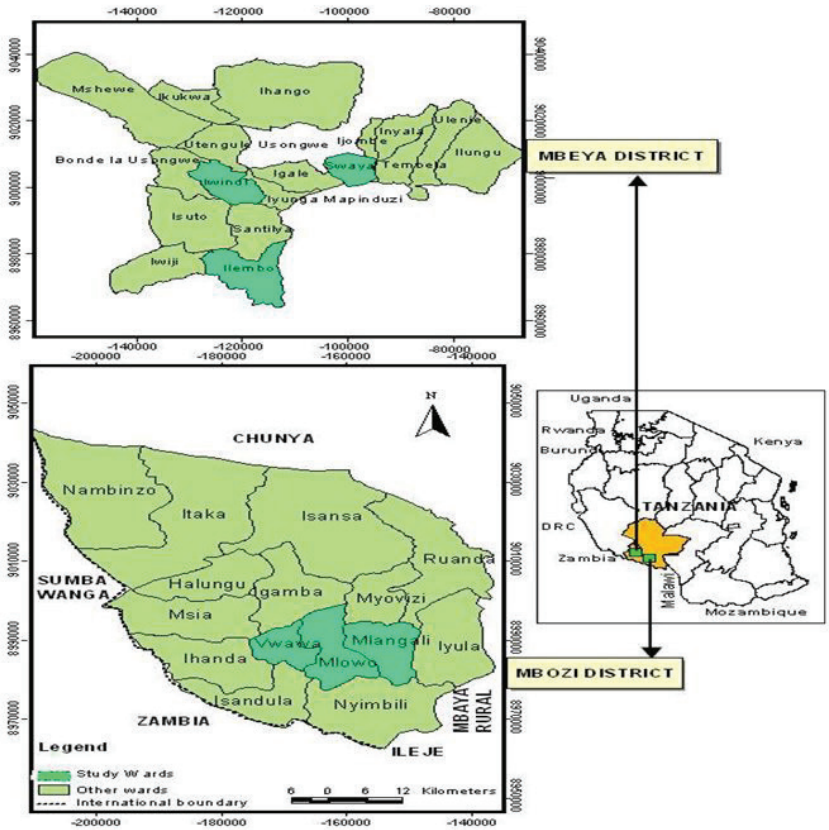

Figure 1: Map of Mbeya region showing studied districts

within Mbeya region (Fig.1). Mbozi district is located in the south western corner of Mbeya region. The district is located between latitudes 8° and 9°12' S and longitudes 32° 7'30" and 33° 2 0'East. The rainfall pattern in the district is unimodal and the rainy season starts from November to May, while the dry season starts from June to October. Annual rainfall ranges from 800 mm to 1 100 mm. Annual temperature ranges between 16°C and 23°C (URT, 1997). Mbeya rural district lies between latitude 8°38' and 9°20'S and longitude 33o and 33°52'E. The rainfall pattern in Mbeya district is unimodal, which ranges from 800 mm to 1 800 mm annually. Annual temperature ranges between 16°C and 20°C (URT 1997). Wet and dry seasons are the same as in the Mbozi district.

Study design and sampling procedure

A cross-sectional study was used to gather information on management and milking practices done by dairy farmers in the study area. Samples were collected from farmers' bulk herd milk to determine the TBC, TCC and TSC. The sample sizes for households which keep dairy cattle and milk samples for microbiological analysis were calculated by using the formula described by Fox *et al.,* (2007). The household sample size was based on proportion of dairy farmers (4%) in the two districts (URT, 2012). The sample size for milk was based on prevalence of 20.4 % of *S. aureus* reported in Tanzania (Mdegela *et al.,* 2004). Sample size was determined at 95% confidence interval and 5% margin for random error where N = P (100% - P)/ (SE) 2, SE = MRE/1.96.

Where; N = Sample size, P = Prevalence of subclinical mastitis, SE = Standard error, MRE = Margin for random error (5%) and 1.96 = tabular value for 95% confidence interval.

From above formula, sample size was as follows; 96 herd milk samples and the criterion for household inclusion in the sampling frame was any household with at least one lactating dairy cow during the study period.

Sampling procedure

Twelve villages were randomly selected by using a table of random numbers, from a sampling frame comprising a list of all villages in the study area, which were obtained from the District Livestock office. Within each village eight households were randomly selected for milk sampling. Structured questionnaire, checklist and observation were used to collect information from randomly selected households in order to link the information recorded with the milk quality. Information on milking hygiene practices, milking place, milk handling, milking time, cowshed (barn), floor type and constraints in producing quality milk were recorded. Other data include household head education level, herd size, economic activities, access to training and the experience in livestock farming.

Samples were collected between 6.15 am and 7.30 am, and 4.15 pm and 6.30 pm from farmers herds, milk collection points and milk shops. Samples were collected in sterile falcon tubes of 50 ml with a screw lid and kept in a cool box at less than 4°C and then transported to the Tanzania Livestock Research Institute (TALIRI), Uyole, and stored at -20°C. The next day after morning collection, samples were transported to Sokoine University of Agriculture (SUA) and the microbiological analysis was performed within 36 hrs.

Microbiological examination of samples

In the laboratory, enumeration of TBC, TCC and TSC were performed by using standard procedures. Briefly, tenfold dilution of each milk sample was prepared using peptone water. Initially dilution of 10^{-2} to 10^{-10} was used in order to establish best dilution to be used in the following samples. The dilution of 10^{-2} to 10^{-7}, 10^{-2} to 10^{-5} and 10^{-2} to 10^{-6} was found appropriate for the best counts for TBC, TCC and TSC, respectively. For the determination of TBC, 0.1 ml of each dilution was transferred using sterile pipette and spreaded on Plate Count (PC) agar (Oxoid, UK) using a sterile glass spreader for each sample. The plates were then kept in an incubator at 30°C for 24-48 hours. Following incubation, colonies were counted with the aid of colony counter. Total Coliform count and TSC were also determined by following the same procedure of TBC except the type of agar and incubation temperature. MacConey agar (HiMedia, Mumbai, India) and Manitol salt agar (Oxoid, UK) were used for TCC and TSC, respectively and incubation temperature was 37°C for 24-48 hr.

Statistical analysis

Descriptive statistics for variables was computed for survey data, which included determination of frequency and percentages. Then a logistic regression analyses in two stages was conducted. In the first stage, the dependent variables (TBC, TCC and TSC) were related to each explanatory variable by means of univariate analysis. In a second stage a logistic regression was conducted. Only variables associated with the outcome variables ($p < 0.10$) were included in the final analysis. Variables that showed a significant association with the log CFU/ml of the three types of microorganisms were analyzed with ANOVA or the Student's T test using Statistical Analysis System (SAS) (2002) software.

The model below was used

$$Y_{ijkl} = \mu_i + H_i + U_j + T_k + P_l + \square_{ijkl} \text{ ..... } I$$

Where;

Y_{ijk} = TBC, TCC, TSC of ith system in jth housing, kth utensil type, tth milk hygiene practices and lth seasons.

μ= overall mean common to all observation

H_i= the effect of ith house floor (where 1= concrete floor, 2=Timber floor, 3= Earth floor, 4= brick wall, 5= timber wall)

U_j= the effect of jth utensil type (plastic or Aluminuium)

T_k = the effect of kth type of milking hygiene practices (where 1= use of individual towel,

water, milking & salve, 2= use of group cow towel and 3= none),
P_l = the effects of lth seasons (wet & dry) and
$\square_{ijkl}$= random error term

Results

Management and milking practices

Table 1 shows the dairy infrastructure in the study area. Common types of cow barn in the study area were those with walls made from timber (65.6%), bricks (burnt/earthen) (34.4%) and floor made from concrete (30.2%), timber (39.6%) and earth/soil (30.2%). Most of the farmers (68.7%) in the study area cleaned the dairy house twice per day and 87.5% milking process was conducted in the cow barn.

The procedures followed during milking in the study area are shown in Table 2. The results show that 96.8% of the respondents washed their hands before milking. Additionally, 57.8% used one piece of towel to the dry udder and teats for many cows. Common types of lubricant used for milking were milking salve (38.5%) and cooking oil (31.2%). Fore milking was conducted by 18.3% of the farmers in the study area. Furthermore, all farmers in the study area did not dip or disinfect the teats before and after milking. Cooling of milk was practiced by 17.7% of the respondents. Water was commonly used to cool milk, mostly evening milk. Wells were found to be the main (47.9%) source of water used in the dairy activities in the study area and most of the respondents (83.3%) used warm water to wash udder and teats before milking.

Factors associated with bacterial count

Variables associated with significant total bacteria counts were season ($p< 0.0001$), frequency of dairy barn cleaning ($p< 0.0001$), cleaning of the cows before milking ($p< 0.001$), cleaning of the udder before milking ($p<0.0001$), wipe udder dry after washing ($p<0.017$), cleaning of milking area ($p<0.02$), types of floor ($p<0.0001$), water source ($p<0.02$), hand washing before milking ($p<0.007$) and herd size ($p<0.006$) (Table 3).

Total coliform counts (TCC): the variables associated with TCC were season ($p<0.0001$), frequency of dairy house ($p<0.001$), cleaning of the cows ($p<0.001$), cleaning of the udder before milking ($p< 0.005$), and water source ($p <0.03$) (Table 3).

Total staphylococcus counts (TSC): Variables associated with significant counts were frequency of cowshed cleaning ($p<0.002$), cleanness of the cows ($p<0.023$), cleaning of the udder ($p<0.005$), personal cleanness ($p<0.023$), wipe dry udder after wash ($p<0.003$), cleanness of milking area ($p < 0.001$), type of floor ($p< 0.0001$), hand washing by milker before milking ($p< 0.04$) and herd size ($p<0.031$) (Table 3).

Variables associated with mean Total bacteria counts are shown in Table 4. Wet season had significantly ($p<0.05$) higher TBC than dry season. Frequency of cowshed cleanness had significantly influence on TBC. Cleaning of the cowshed 2 to 3 times per day significantly ($p< 0.05$) reduce TBC than those cleaning once per day. Furthermore, milk collected from farmers whose dry the udder with single used towel per cow significantly ($p<0.04$) lower TBC than those use single towel for many cow. Cow barn made from concrete floor had milk with significantly ($p<0.001$) lower TBC than that made from timber and earth floor. Herd which had few cows produced milk with significantly ($p<0.05$) lower bacteria counts than herd with many cows.

Table 5 shows the variables significantly associated with mean TCC. The use of well water significantly ($p<0.03$) influenced TCC than other sources. Milking out of cow barn had significantly ($p< 0.05$) lower TCC than inside the cow barn. Milk samples obtained from farmers who washed udders and teats before milking had significantly ($p< 0.05$) lower TCC than those from farmers who did not carry out this practice.

Variables associated with mean TSC are shown in Table 6. Frequency of cow barn cleaning two to three times per day had milk with significantly ($p<0.05$) lower TSC than single cleaning per day. Dirty cows produced milk with significant ($p<0.05$) higher TSC counts than clean cows. Drying udder with individual cloth per cow produce significantly

Table 1: Types of cowshed structure and management practices

Parameter	District			P- value
	Mbeya (n=48)	Mbozi (48)	Total (n=96)	
Type of wall	%	%	%	
Burnt bricks	20.8	25	22.9	
Unburned bricks	10.4	12.5	11.5	0.812
Timber	68.8	62.5	65.6	
Type of roof				
Closed	52.1	81.2	66.7	
Open	4.2	0	2.1	0.005
Half closed	43.8	18.8	31.2	
Type of floor				
Concrete	22.9	37.5	30.2	
Timber	45.8	33.3	39.6	
Earth	31.2	29.2	30.2	0.17
Frequency of cowshed cleaning				
Once per day	12.5	24	18.3	
Twice per day	75	62.5	68.7	
Thrice per day	12.5	13.5	13	0.03
Milking place				
Inside the cowshed	85.4	89.6	87.5	
Crush	2.1	8.3	5.2	
Open space	12.5	2.1	7.3	0.03

Table 2: Procedure followed during milking among smallholder dairy farmers

Parameter	Level	District		Total	P value
		Mbeya(n=48)	Mbozi (n=48)	(n=96)	
Hand wash before milking	Yes	97.9	95.8	96.8	0.001
Material used to dry udder	Towel	20.8	29.2	25	
	Piece of cloth	35.4	47.9	41.7	
	No drying	43.8	22.9	33.3	0.17
Application of drying material	One piece per cow	37.0	63.0	42.2	
	One piece to all cow	45.9	54.1	57.8	0.001
Source of water used for washing	Spring	4.2	8.3	6.2	
	Tap	29.2	10.4	19.8	
	River	16.7	2.1	9.4	0.002
	Well	25	70.8	47.9	
Teat lubrication	Yes	91.7	93.8	92.7	
Type of lubricant	Milking salve	31.2	45.8	38.5	
	Cooking oil	33.3	29.2	31.2	
	Body lotion	27.1	18.8	22.9	

Parameter	Level	District		Total	P value
		Mbeya(n=48)	Mbozi (n=48)	(n=96)	
	Milk	8.3	6.2	7.3	0.07
Water for washing teats	Warm	72.9	93.8	83.3	
	Cold	25	2.1	13.5	
	Mixture (Warm & cold)	2.1	4.2	3.1	0.006

Table 3: Association between management and milking practices with TBC, TCC and TSC in bulk herd milk

Variable	TBC	TCC	TSC
Season	<.0001	<.0001	0.1716
Frequency of barn cleaning (once/day, twice/day and thrice/day	<.0001	0.0012	0.0025
Cleanness of the cow (clean, moderate and dirty)	0.001	0.001	0.0254
Cleanness of the udder (clean, moderate and dirty)	<.0001	0.005	0.1391
Personal cleanness	0.0072	0.071	0.023
Experience	0.0792	0.006	0.202
Wipe dry after wash (one towel/cow and common towel)	0.0173	0.440	0.0031
Presence of milking area	0.0321	0.027	0.1426
Cleanness of the milking environment (clean, moderate and dirty)	<.0001	0.523	<.0001
Type of floor (concrete, timber and earth)	<.0001	0.636	<.0001
Wall type (burnt bricks, earth bricks and timber)	0.0156	0.775	<.0001
Water source (tap, well, river and spring)	0.0204	0.013	0.4210
Hand wash before milking (cold water, warm water , mixture and none)	0.0073	0.553	0.0445
Fore stripping (Yes, No)	0.002	0.412	0.032
Herd size (1-3, 4-6 and >7)	0.006	0.071	0.0314

($p<0.006$) lower TSC than using one cloth for many cows. Milk samples collected from cows kept in shed made from concrete floor had significantly ($p<0.05$) lower TSC than from timber and earthen floor. Samples from non hand washers before milking time yielded significantly ($p< 0.05$) higher TSC than those who washed hands.

Correlation of some of management, milking and milk handling practices with bacterial counts are shown in Table 7. Most of the factors showed moderate to low correlation with bacterial counts in the milk. Only two factors (cleaning frequency and type of floor) showed highly significant ($p<0.0001$) strong correlation with TBC. Furthermore, TBC was moderately correlated with cleanness of the cows, cleanness of the teat, cleanness of the cow barn, drying of the teat and types of material used to dry teat. Regarding TCC and TSC, the tested parameters were either moderately, weakly or negatively correlated (Table 7).

Discussion

The cleanliness of the udder and teats prior to milking preparation was associated with TBC, TCC and TSC. The results showed that dirty udders were associated with higher bacteria count in the bulk herd milk. This is because the udder of the cows if highly contaminated or has been insufficiently prepared before milking could contaminate the bulk milk easily. Also the numbers of cows with dirty udders and teats in the herd had significant influence in TBC of bulk herd milk and affected the efficiency of udder preparation.

Table 4: Variables associated with mean log Total bacteria counts in farmers bulked milk

Parameter	Variable	meanlog10cfu/ml
Season	Wet	6.57[b]
	Dry	5.28[a]
Frequency of barn cleaning	Once/day	5.97[b]
	Twice/day	4.08[a]
	Thrice/day	3.95[a]
Cleanness of the cow	Clean	3.47[b]
	Moderately clean	5.45[a]
	Dirty	6.62[a]
Cleanness of the udder	Clean	3.55[b]
	Moderately clean	5.21[a]
	Dirty	6.07[a]
Wipe dry after wash	Single towel/cow	4.67[b]
	One towel/many cow	6.40[a]
Cleanness of the milking environment	Clean	4.77[b]
	Moderately clean	6.38[a]
	Dirty	6.59[a]
Type of floor	Concrete floor	5.12[b]
	Timber floor	6.43[a]
	Earth floor	6.63[a]
Water source	Tap	5.58[c]
	Well	6.04[b]
	River	6.34[a]
	Spring	6.01[b]
Personal cleanness	Clean	5.56[b]
	Moderately clean	6.42[a]
	Dirty	6.70[a]
Hand wash before milking	Yes	5.17[b]
	No	6.70[a]
Fore striping	Yes	4.15[b]
	No	5.94[a]
Herd size	1-3	4.35[b]
	4-6	5.18[b]
	>7	6.21[a]

Means with the same superscript within a column do not differ significantly (P > 0.05)

Table 5: Variables associated with mean log Total coliform counts in farmers bulk herd milk

Parameter	Variable	meanlog10cfu/ml
Season	Wet	5.20[a]
	Dry	2.37[b]
Frequency of barn cleaning	Once/day	5.08[a]
	Twice/day	4.12[b]
	Thrice/day	3.36[b]
Cleanness of the cow	Clean	3.02[c]
	Moderate	4.24[b]
	Dirty	4.92[a]
Cleanness of the udder	Clean	2.98[c]
	Moderate	4.12[b]
	Dirty	4.86[a]
Water source	Tap	3.15[b]
	Well	3.86[a]
	River	4.28[a]
	Spring	3.67[a]
Experience of keeping livestock	1-10	3.92[a]
	11-20	3.78[a]
	21-30	3.10[b]
	>31	3.02[b]
Hand wash before milking	Yes	3.79[a]
	No	3.77[a]

Means with the same superscript within a column do not differ significantly (P > 0.05)

Table 6: Variables associated with mean log Total staphylococcus counts in farmers bulk milk in Mbeya, Tanzania

Parameter	Variable	meanlog10cfu/ml
Season	Wet	4.85[a]
	Dry	4.79[a]
Frequency of barn cleaning	Once/day	5.23[a]
	Twice/day	4.12[b]
	Thrice/day	4.03[b]
Cleanness of the cow	Clean	3.41[b]
	Moderate	3.47[b]
	Dirty	4.98[a]
Wipe dry after wash	Single towel/cow	3.78[b]
	One towel/many cow	5.14[a]
Cleanness of the milking environment	Clean	4.03[b]
	Moderate	4.98[a]
	Dirty	5.35[a]
Type of floor	Concrete floor	4.03[c]

Parameter	Variable	meanlog10cfu/ml
	Timber floor	5.11[b]
	Earth floor	5.95[a]
Hand wash before milking	Yes	4.01[b]
	No	5.41[a]
Personal cleanness	Clean	4.12[b]
	Moderate	5.35[a]
	Dirty	5.55[a]
Herd size	1-3	3.25[b]
	4-6	4.98[a]
	>7	5.29[a]

Means with the same superscript within a column do not differ significantly (P > 0.05)

The milk from cows that had dirty udders and teats before milking had significantly higher bacteria counts than cows with clean udders and teats. Similarly milking procedures and types of udder preparation had significant effects on the bacteria count. Previous studies by Ellis *et al.,* (2007) had reported a positive association between the degrees of udder contamination and microbial counts. Also findings by Elmoslemany *et al.,* (2009) showed a positive association between udder hygiene score and bacterial counts in bulk tank milk.

Clean and appropriate floor for milking cows is important for the production of good quality milk. One of the objectives of cleaning cow barn is to make cows clean and eventually milk cows in clean environment. In this study the type of floor significantly influenced the bacterial quality of milk. Concrete floors were significantly associated with lower TBC, TCC and TSC where as earth/soil floor had significantly higher TBC, TCC and TSC. Cow teats could have variable contamination level depending on the place where animals were kept before milking and climate conditions. It was observed in the present study that cows kept in earthen floor had dirty flanks, abdomen, udder and teats and hygiene was worse during the wet season compared to the dry season. Milking in such an area could result into high bacteria counts in the milk and negatively affect its keeping quality because wet skin is known to shed more bacterial colonies than dry skin. Proper and clean milking environment is a requirement for quality milk of acceptable microbial level and longer shelf life. On the other hand earth and timber floor was associated with increased TBC and TSC in the bulk herd milk. This is because in earth floor the teats and udder often become soiled with manure and urine due to difficulty of cleaning and inefficient drainage. In the case where the teats are not properly cleaned and dried before milking, all dirt and associated microorganisms in it will contaminate the milk. Similar findings were reported in other studies conducted in Mvomero and Njombe districts (Chang'a *et al.,* 2008), Sri Lanka (Vairamuthu *et al.,* 2010) and Kenya (Kembe and Omondi, 2015).

In this study cleaning frequency of cow's barn two to three times per day was associated with the lowest TBC, TCC and TSC compared to single cleaning per day. The frequency of cow barn cleanness affects the cleanness of the udder, teats and adjacent parts (flanks, hind quarters, tails and abdomen). It was found that lower frequency of cow barn cleaning was associated with dirty cows and higher bacterial counts. This is because the extent of soiling of the udder and teat surface depends on the cleanness of the cow barn and the time required for udder preparation increase in case of dirt cows, which may have influenced milking efficiency and may have led to inadequate preparation of the udder (Ellis *et al.,* 2007).

Generally, the hand, udder and teat wash were performed by most farmer using

Table 7: Pearson correlation for some of management, milking and milk handling practices with bacteriological quality

	TBC	TCC	TSC	PCNES	CBAN	CCOW	CUDR	DDR	FQCL	CLME	TPFL	HW	WS	FM	HS
TBC		0.179**	0.522***	0.319***	0.467***	0.475***	0.489***	0.251*	0.594***	0.443***	0.621***	0.462***	0.235**	0.466***	0.296**
TCC			-0.044*	0.304**	0.369***	0.356**	0.321**	0.343**	0.281*	0.172*	0.354**	0.291**	0.292**	-0.020	0.300***
TSC				0.219*	0.182*	0.193*	0.169*	0.082NS	0.493***	0.270**	0.462***	0.341**	0.187*	0.311***	-0.019^{NS}
PCNES					0.569***	0.523***	0.452***	0.043NS	0.239**	0.216**	0.325***	0.642***	0.021^{NS}	-0.043^{NS}	0.032^{NS}
CBAN						0.894***	0.705***	0.012NS	0.352***	0.334***	0.382***	0.017^{NS}	0.001^{NS}	-0.018^{NS}	0.564***
CCOW							0.625***	0.046NS	0.357***	0.343***	0.376***	0.022^{NS}	-0.023^{NS}	0.024^{NS}	0.341***
CUDR								0.173*	0.434***	0.466***	0.481***	-0.011^{NS}	0.056^{NS}	0.102^{NS}	0.194*
WUDR									0.143*	0.176*	0.253**	0.126^{NS}	0.113^{NS}	0.121^{NS}	0.110^{NS}
FQCL										0.485***	0.568***	-0.032^{NS}	0.109^{NS}	0.013^{NS}	0.301***
CLME											0.583***	-0.025^{NS}	0.037^{NS}	-0.083^{NS}	0.192*
TPFL												0.081^{NS}	-0.017^{NS}	-0.008^{NS}	0.233**
HW													0.134^{NS}	0.168*	0.096^{NS}
WS														0.112^{NS}	0.021^{NS}
FM															0.129^{NS}

*TBC-Total bacteria count, TCC- Total coliform count, TSC- Total staphylococcus count, PLNES-Personal cleanliness, CBAN- Cleanliness of barn, CCOW- Cleanliness of cow, CUDR- Cleanliness of the udder, DDR-Drying udder after wash, FQCL-Frequency of barn cleanliness, CLME- Cleanliness of milking environment, TPFL- Type of barn floor, HW-Hand wash before milking, WS-Water source, FM- Fore milking, HS-Herd size. ***significance (P<0.0001), **(P<0.001), *(P<0.05), NS- Not significant(P>0.05).*

warm water without any disinfectant or detergent. The possibility of contamination from the milker is higher in the case of hand milking if the hands were not effectively washed. In the present study, herds where washing hands, udder and teats and followed by drying of teats by individual towel contained significantly lower TBC and TSC than the use of one towel for many cows. Similar finding was reported by Molineri *et al.,* (2012) that the drying of the teats with single towel associated with the lowest bacterial counts compared to other methods. Pandey & Voskuil (2011) underlined the importance of hygienic milking preparation protocol that hands should be washed with clean water and soap and dry them with a clean towel before milking and during the milking procedure between each cow. Also Vissers *et al.,* (2007) found that teat washing and drying before milking generated lower contamination on teats and lower total bacterial counts than washing only or not washing at all. Herd size significantly influences bacterial count; bulk herd TBC and TSC were lower in herds with few cows than herds with many cows. The reason for higher bacteria count in herd with many cows could be the use of water and drying towel was not changed between milking cows, thus contaminate the bulk herd milk. Similar finding was reported by Jayarao *et al.,* (2004) that herd size and farm management practices influenced bacterial count in bulk tank milk.

Fore-milking improves the quality of milk by allowing the milker to see any changes in the milk and removing the first strips of milk that contain high bacteria counts. In the present study most farmers did not practices this procedure which has great influence in bulk herd milk quality. Lack of awareness was among the reason as to why farmers not performing this practices. Study conducted in Mvomero and Njombe districts by Chang'a *et al.,* (2008) reported the importance of this practice in the production of milk with lower TBC.

Premilking teat dip and post milking dip of teats in disinfectant were the practices not performed by all of the farmers in the study area. Farmers were not aware on the importance of these practices on the milk quality and prevention of contagious mastitis. Several studies underline the importance of pre and post milking dipping teat disinfectant on the reduction of bacterial counts in the milk. Study by Gibson *et al.,* (2008) reported that the washing of teats with an effective disinfectant and then drying was the most effective method of reducing microbial counts in the milk. Also Ruegg (2004) pointed out that pre-dipping of the teats with an approved disinfectant was considered the most effective way of teat disinfection, and drying of the teats before milking was considered the most important step in a teat-cleaning regime. Following the importance of these practices neither of the farmers in the study area was aware of this practice and their importance in the production of good quality milk and mastitis control. It has been reported that pre-milking teat disinfection by teat dipping in association with good udder preparation reduced the rate of intramammary infections due to environmental pathogens (Karimuribo *et al.,* 2008).

Most of the farmers in the study area kept milk at room temperature. Few of the farmers cooled milk especially evening milk mainly by using cold water. Keeping fresh milk at an elevated temperature together with unhygienic practices during the milking process may result in high microbial count causing inferior milk quality. Several workers (Kurwijila, 2006; Zalalem, 2010; Amakelew *et al.,* 2015) underline the importance of cooling the milk immediately after milking at a temperature of 4oC, the process which prevent the rapid growth and multiplication of bacteria present in the milk. Cooling milk being important practices it is usually neglected by most of the farmers in the study area. This can be due to the lack of awareness and cooling facilities which is a challenge in most of dairy keepers in the Southern highlands zone.

Correlation of some management, milking and handling practices with microbial quality revealed that two practices had strong correlation with TBC, other practices showed moderately correlation with microbial count and most of the practices were weakly

correlated meaning that neither of practices alone had a big influence on increased microbiological count of milk. Several authors (Elmoslemany *et al.,* 2009; Elmoslemany *et al.,* 2010 and Welearegay *et al.,* 2012) pointed out the importance of good milking and handling practices on microbial quality. They pointed out that quality of milk was determined by various aspects of dairy management, milking and handling practices.

Conclusion and recommendation

Milking preparation procedures, the frequency of cow barn cleaning and type of floor were associated with bacteriological counts in raw milk.

Season affected most of the management practices and influenced TBC and TCC.

Most of the practices had low to moderate correlation to milk bacteriological quality. This showed that no single practices can itself contribute higher to the bacterial load of milk. Thus, each procedure involved in pre milking and post milk handling should be dealt independently, hygienically, effectively and farmers must understand why each procedure is being done.

There should be efforts to develop standard milking procedure in the Southern highlands zone with the aim of reducing contamination and improve milk quality at the production level and training farmers in good agricultural practices is of benefit in the study area.

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Competing interests

The authors declare that they have no competing interests.

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EFFECTS OF TRIS-BASED PUMPKIN SEED MILK EXTENDER SUPPLEMENTED WITH VITAMIN C ON SPERM VIABILITY OF WEST AFRICAN DWARF GOAT BUCK DURING COLD STORAGE

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Abstract

The effects of Tris based pumpkin seed milk extender supplemented with Vitamin C on sperm viability after storage was carried out in an *in vitro* study. Semen samples obtained from five (5) intact West African Dwarf (WAD) WAD bucks were pooled together for this study. The pooled semen were divided into 5 equal aliquots and diluted with Tris based pumpkin seed milk (TPSM) extender (20% pumpkin seed milk) supplemented with vitamin C at different levels, (0mM, 2mM, 4mM, 6mM and 8mM) respectively. The diluted semen samples were loaded into tubes, stored in a refrigerator maintained at 5°C for a period of 96 hours followed by evaluation. The result obtained showed higher ($P<0.05$) percentage motility at 4mM, 6mM, and 8mM. Significantly higher percentage acrosome integrity were obtained at 2mM, 4mM, 6mM, and 8mM of vitamin C, while 4mM, 6mM, and 8mM vitamin C supplementation in TPSM sustained membrane integrity compared to the control. Lower ($P<0.05$) abnormal sperm were observed at 2mM, 6mM and 8mM supplementation of vitamin C in TPSM extender. Reduced ($P<0.05$) malondialdehyde (MDA) concentrations were observed with 8mM inclusion level and significantly higher ($P<0.05$) acrosin activity value was observed at 8mM, while the result showed increased ($P<0.05$) arginase activity at 6mM inclusion level of vitamin C compared to the control. Results revealed vitamin C at 6mM and 8mM in TPSM extender had higher ($P<0.05$) percentage *in vitro* acrosome reaction and 8mM inclusion of vitamin C had highest ($P<0.05$) percentage *in vitro* sperm capacitation. The result revealed that optimal improvement in these viability parameters with increased levels of vitamin C supplementation in TPSM extenders. Therefore, supplementation of Vitamin C at higher levels (6mM and 8mM) in TPSM based extenders could sustain sperm viability parameters of WAD goat bucks during cold storage"

Keywords: Vitamin C; Pumpkin seed Milk; Goat buck; Cold storage; Sperm viability

EFFETS DU DILUEUR DE LAIT DE GRAINES DE COURGE À BASE DE TRIS COMPLETE AVEC DE LA VITAMINE C SUR LA VIABILITÉ DU SPERME DES CHÈVRES NAINES D'AFRIQUE DE L'OUEST MALES PENDANT LE STOCKAGE AU FROID

Resume

Les effets du dilueur de lait de graines de courge à base de Tris complété avec de la vitamine C sur la viabilité des spermatozoïdes après le stockage ont été évalués dans le cadre d'une étude in vitro. Des échantillons de sperme obtenus de cinq (5) chèvres naines d'Afrique de l'Ouest (WAD) mâles intacts ont été regroupés pour cette étude. Le sperme regroupé a été divisé en 5 parties aliquotes égales et dilué avec

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dilueur de lait de graines de courge (TPSM) à base de Tris (20% de lait de graines de courge) additionné de vitamine C à différents niveaux (respectivement 0mM, 2mM, 6mM et 8mM). Les échantillons de sperme dilué ont été placés dans des tubes, stockés dans un réfrigérateur maintenu à 5oC pendant une période de 96 heures suivie d'une évaluation. Le résultat obtenu a montré un plus grand pourcentage de mobilité (P <0,05) à 4 mM, 6 mM et 8 mM. Un pourcentage significativement élevé d'intégrité de l'acrosome a été obtenu à 2 mM, 4 mM, 6 mM et 8 mM de vitamine C, tandis qu'une supplémentation en vitamine C de 4 mM, 6 mM et 8 mM dans le TPSM a maintenu l'intégrité membranaire par rapport au témoin. Des spermatozoïdes anormaux inférieurs (P <0,05) ont été observés à 2 mM, 6 mM et 8 mM de supplémentation en vitamine C dans le dilueur TPSM. De faibles (P <0,05) teneurs en malondialdéhyde (MDA) ont été observées au taux d'inclusion de 8 mM et une activité de l'acrosine significativement plus élevée (P <0,05) a été notée au niveau d'inclusion de 8 mM, tandis que le résultat a montré une activité arginase accrue (P <0,05) au taux d'inclusion de 6mM de vitamine C par rapport au témoin. Les résultats ont révélé que la vitamine C à 6mM et 8mM dans le dilueur TPSP avait un pourcentage plus élevé (P <0,05) de réaction acrosome in vitro, et l'inclusion de 8mM de vitamine C avait le plus haut (P <0,05) pourcentage de capacitation in vitro des spermatozoïdes. Le résultat a révélé une amélioration optimale de ces paramètres de viabilité avec des niveaux accrus de supplémentation en vitamine C dans les dilueurs TPSM. Par conséquent, la supplémentation en vitamine C à des niveaux plus élevés (6 mM et 8 mM) dans les dilueurs à base de TPSM pourrait soutenir les paramètres de viabilité des spermatozoïdes des boucs WAD pendant le stockage au froid.

Mots-clés : Vitamine C ; lait de grains de courge ; chèvre mâle ; stockage au froid ; viabilité du sperme

Introduction

West African Dwarf (WAD) goats (Capra hircus) possess certain valuable traits that confer adaptation to hot humid tropics (Daramola and Adeloye, 2009). This animal serves as one of the means of livelihood for residents of the rural environments in southern Nigeria. Due to increased urbanization and industrialization in Nigeria, human population in Nigeria is becoming increasing resulting in higher demand for animal protein thereby putting this important breed of goat into danger of extinction if drastic measures are not put in place. Preservation of semen from this goat breed via cold storage (Daramola and Adekunle, 2015) can be harness to improve its reproductive advantage prior to artificial insemination. Mammalian sperm is susceptible to damage and deterioration by excess reactive oxygen species (ROS) during storage because it contains high amounts of polyunsaturated fatty acids (Aitken and Fisher, 1994). Decline in motility, membrane integrity and fertility of mammalian spermatozoa has been observed when stored at 5°C (Maxwell and Salamon, 1993; De Lamirande *et al.,* 1997). Like spermatozoa from other mammals, goat spermatozoa normally contain antioxidants which may not be sufficient to prevent lipid peroxidation during prolonged storage (Aurich *et al.,* 1997) and are not sufficient to support cell activities during *in vitro* storage. The common component of most semen preservation extenders used for domestic animals is Egg-yolk (EY) and it has been shown to have beneficial effects on sperm preservation as a protectant against temperature (Su *et al.,* 2008). However, EY has been found to represent certain problems because it contains micro elements that might be responsible for increasing extender's viscosity, microbial harbouring, and inhibition sperm respiration thereby declining sperm motility (Sharafi *et al.,* 2009). A specific problem limiting the storage properties of goat semen is the presence in seminal plasma, an enzyme derived from bulbo-urethral glands, that coagulate egg yolk (egg yolk coagulating enzyme - EYCE) (Leboeuf *et al.,* 2000) which was discovered to be higher at some breeding season Ferreira et al. (2014). EYCE is characterized as phospholipase A which hydrolyses egg yolk lecithin to fatty acids and lisolecithin. Lisolecithin is toxic to goat spermatozoa which constitute a major disadvantages of EY extender. Plant source

milk has recently gain scientist attention as a potential replacement for animal source protein extenders because of the richness of plant source milk to protect spermatozoa during storage, and the protective effects of adding antioxidants such as Vitamins C, E, B6 and melatonin to semen extender has been reported (Daramola *et al.,* 2016). Fluted pumpkin (*Telfairia occidentalis*) is one of the most widely cultivated leaf vegetables in southern Nigeria, although the major area of cultivation is the south-eastern Nigeria (Ogar and Asiegbu, 2005).The tender vine and foliage are consumed as pot herbs while the seed is consumed as nut. Protein and oil contents of the seed are 30.1 % and 47 % respectively (Asiegbu, 1987) and it has been reported that the seed is of good nutritional values (USDA, 2016). Like coconut milk and soya milk, another plant source milk that can be harness for its potential to preserve goat sperm is pumpkin seed milk which has been noted to contain minerals and nutrients (USDA, 2016) required for sperm survival during storage but no information have been reported in literatures. Therefore, the present study evaluated the effects of tris pumpkin seed milk based extender supplemented with vitamin C on WAD goat buck spermatozoa during cold storage.

Materials and Methods

Preparation of Pumpkin Seed Milk (PSM)

Fluted Pumpkin fruit was sourced from a local market. The method of milk extraction from pumpkin seed was adapted from the work of Rehman *et al.,* (2014) and the procedures with some modifications are as follows: the fruit was split opened with knife to retrieve the seed, and the weight of the seeds when weighed was between 33-35 grams. The outer coat of the seeds were removed and later transferred into boiling water for 10 minutes to soften. The softened seeds were then chopped and poured into an automatic blender to blend the seed. After blending, 500mL of clean water was added to the slurry, mixed thoroughly and then sieved with a new clean white sieve clothe. The milk extract was then transferred into a beaker ready for use.

Semen Collection, Dilution and Storage

Semen samples were collected from five bucks using artificial vagina. A total of five semen samples (each semen sample originating from the five bucks) showing >80% motility were pooled to minimize individual differences (Bucak and Tekin, 2007). The composition of semen extender used in this study consisted of Tris-hydroxymethyl-aminomethane (2.42 g), citric acid (1.35 g), glucose (1 g), penicillin (0.028 g), 20 ml pumpkin seed milk (PSM) and distilled water made up 100ml. The pooled fresh semen was then splitted into five equal fractions in different test tubes and diluted with the extenders supplemented with varying levels of Vitamin C at 2mM, 4mM, 6mM, and 8mM/100mL of the diluents respectively and the control with zero supplementation of Vitamin C and a final concentration of 373 x 106 sperm / ml. The pH of the extender (control 7.03; Vitamin C supplements: 7.09 - 7.12) was determined using a digital pH meter. Following dilution, the diluted semen samples were sealed and chilled from 37°C to 5°C at approximately 0.5°C/ min and maintained at this temperature in a refrigerator for 96 hours and thereafter evaluated for sperm quality characteristics.

Semen Evaluation

Sperm progressive motility:

Sperm motility was determined as described by Bearden and Fuquay (1997). Briefly, semen was warmed in Clifton Water bath (Model: 74178 by Nickel Electro Ltd, Weston-S-Mare Somerset, England) at 37°C and observed for sperm motility using Celestron PentaView compound microscope (LCD-44348 by RoHS, China) at 400 x magnification. A 5µL sample of semen was placed on a heated microscope slide and overlaid with a 22 x 22 mm cover slip. For each sample, ten microscopic fields were observed for sperm progressive motility by two observers and the mean of the ten successive evaluations was recorded as the final motility score.

Acrosome integrity:

The percentage of spermatozoa with intact acrosome(s) was determined according to (Ahmad *et al.,* 2003) and (Ahmad *et al.,* 2014). Briefly, 50µL of each semen sample was added to a 500µL formalin citrate solution (96 ml 2.9% sodium citrate, with 4ml 37% formaldehyde) and mixed carefully. A small drop of the mixture was placed on a microscope slide and a total of 200 spermatozoa were counted in at least four different microscopic fields for each sample, using a Celestron PentaView LCD compound microscope (400 x magnification). Intactness of acrosome characterized by normal apical ridge of 200 spermatozoa were observed and recorded.

Membrane integrity:

Hypo-osmotic swelling test (HOST) was used to determine sperm membrane integrity (Jeyengran *et al.,* 1884). This was done by incubating 10 µL semen in 100µL Hypo-osmotic solution (7.35g sodium citrate (0.0285M) and 13.5g fructose [0.075M]) at 37°C for 30 minutes. 0.1ml of the mixture was spread over a cover slip warm slide and observed under a Celestron PentaView LCD compound microscope (400 x magnification). 200 spermatozoa were counted and the percentage of spermatozoa positive to HOST for their swelling characterized by curled tails, indicating intact plasma membrane was determined and those with no swelling characterized by uncurled tails were classified as spermatozoa with abnormal membrane integrity.

Abnormality:

Sperm morphological abnormalities were determined as described by Bearden and Fuquay (1997), with the use of eosin-nigrosin smears. Briefly, a thin smear of mixture of semen and eosin-nigrosin solution was drawn across the slide and dried. The percentage of morphologically abnormal spermatozoa with defects in the head, midpiece and tail were observed under a Celestron PentaView LCD compound microscope (400 x magnification).

Malondialdehyde (MDA) concentrations:

Malondialdehyde (MDA) concentration as index of lipid peroxidation in the stored semen was measured in a thiobarbituric acid reactive substances (TBARS) according to Pipan et al. (2014). For this assay, 0.1 mL of sperm suspension was incubated with 0.1 mL of 150 mM Tris–HCl (pH 7.1) for 20 min at 37oC. Subsequently, 1 mL of 10% trichloroacetic acid (TCA) and 2 mL of 0.375% thiobarbituric acid were added and incubated in boiling water for 30 min. Thereafter, it was centrifuged for 15 min at 3000 × g inside blank tube and the absorbance was read with UV spectrophotometer (SW7504 model by Surgifriend Medicals, England) at 532 nm. The concentration of MDA was calculated as follows: concentration of malondialdehyde MDA (nmol/mL) = AT – AB/1.56 × 105; where AT = absorbance of the sample, AB = absorbance of the blank, and 1.56 × 105 = molar absorptivity of MDA.

Arginase activity:

Arginase activity was spectrophotometrically determined using the protein concentration method described by Lowry et al. (1951). Briefly, 0.1 g bovine serum albumin (BSA) in10 mL of water was used as standard. Alkaline copper reagent (1 mL) was prepared as follows: Copper sulfate reagent (100 mg of cupric sulfate + 200 mg of sodium tartrate in 50 mL distilled water + 10 g of sodium carbonate in 50 mL distilled water), sodium dodecyl sulfate solution (5 g of sodium dodecyl sulfate in 100 mL distilled water), and sodium hydroxide solution (3.2 g of sodium hydroxide in 100 mL distilled water) were mixed in ratio of 1:2:1. The alkaline copper reagent (1 mL) and semen samples (0.1 mL) were mixed in tubes and incubated for 10 minutes at room temperature. Thereafter, 4 mL folin Ciocalteu's phenol reagent was added to the tubes, mixed and incubated for 5 min at 55°C. The absorbance of the samples was recorded at 650 nm using a spectrophotometer (UV spectrophotometer, SW7504 model by Surgifriend Medicals, England). Values obtained were expressed as units/mg of protein (specific activity).

Acrosin activity

Evaluation of acrosin activity were carried out according to the procedure described by (Rosatti et al, 2004). BAPNA (N-□- benzoyl-DL-arginine p-nitroanilide) was used as a specific substrate for acrosin. A solution of HCl (10 M) was used to inhibit activation of proacrosin to acrosin. A solution of HCl was added to a control which was incubated at 38 °C. Aliquots was taken from each sample at a time and incubated. Again, a solution of HCl was added to all the samples, except for the control and the total acrosin ones. Samples was homogenized and centrifuged at 14,000 xg for 10 minutes. A solution of 0.2 M buffer Tris and 100 µL of BAPNA (100 mM) was added to each supernatant, which were then incubated for 3 minutes at room temperature. Sample absorbance (Ab) was recorded at 410 nm. Activity was expressed in mIU/106 sperm and acrosin activity was calculated as follows:

mIU of acrosin/10^6 sperm = [Ab sample – Ab control] x 10^6/9.9 mM^{-1}x cm^{-1} x 3 minutes x 10^6 sperm x vol. of cuvette.

In vitro acrosome reaction:

Following cold storage, spermatozoa were evaluated for *in vitro* acrosome reaction using the method described by Tardif et al. (1999) with modification as follows: Samples of the cooled semen were washed by centrifuging at 3000 × g for 5 minutes with non-culture medium phosphate buffered saline (PBS), and the pellets were resuspended in culture medium (Calcium chloride di hydrate 265 mg/L, Magnesium chloride anhydrous 46 mg/L, Potassium chloride 200 mg/L, Sodium chloride 8000 mg/L, Sodium dihydrogen phosphate anhydrous 50 mg/L and D-glucose 1000 mg/L). Following the addition of 0.9% wt/vol PBS, acrosome reaction was induced by incubating spermatozoa for 20 minutes with progesterone (2.5 mg/mL) at 38.5°C (5% CO2t in air; 100% humidity). To determine the proportion of spontaneous acrosome reaction, progesterone was omitted but an equal volume of PBS was added. Then, 100 sperm cells were counted per slide in an upright Carl Zeiss Fluorescent Microscope (PrimoStar, Germany) equipped with phase contrast and epifluorescence optics. Spermatozoa with intense fluorescence over the acrosome were classified as acrosome intact and those with no fluorescence or a dull fluorescence along the equatorial segment as acrosome that underwent reaction.

In vitro capacitation:

In vitro capacitation of the spermatozoa was evaluated using the chlortetracycline (CTC) fluorescence assay as described by Collin *et al.,* (2000). In brief, CTC (750µM) was prepared in 20 mM Tris buffer containing 130mM NaCl and 5mM dl-cysteine (final pH = 7.8). Semen suspension (5µL) was mixed with 5µL of CTC solution on a warmed slide (37^0C). After 30 s, 5µL of 0.2% glutaraldehyde in 0.5 M Tris (pH = 7.4) was added. Finally, 5µL of 90% glycerol and 10% PBS (pH adjusted to 8.6) were added to retard fluorescence fading. After adding a cover slip, slide was examined with an upright Carl Zeiss Fluorescent Microscope (Primo Star, Germany) equipped with phase contrast and epifluorescence optics, and 100 cells were counted per slide. The spermatozoa which exhibited pattern (characterized by bright anterior head and faint fluorescence in the post acrosomal region) according to the CTC assay were classified as capacitated spermatozoa.

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) using SAS 2000, while Duncan Multiple Range Test (Duncan, 1955) was used to separate significantly different means. The model used is shown below

$Y_{ij} = \mu + L_i + \sum_{ijk}$

Where, Y_{ij} = Dependent variables;

µ= Population mean;

Li = effect due to ith level ofVitamin C inclusion, i = (0, 2, 4, 6, 8)

$\sum_{ijk}$ =Random error

Table 1: Means (±SEM) motility and structural integrities of WAD buck spermatozoa preserved with PSM extender supplemented with Vitamin C during cold storage.

Parameters	Inclusion levels of Vitamin C in PSM extender				
	0mM	2mM	4mM	6mM	8mM
MOT (%)	48.00±2.905^{b}	56.40±3.208^{b}	74.60±2.413^{a}	68.70±6.496^{a}	73.00±4.725^{a}
ACI (%)	55.00±2.880^{b}	78.50±2.500^{a}	80.00±2.440^{a}	81.00±1.730^{a}	84.50±0.500^{a}
MI (%)	56.00±2.440^{c}	77.00±1.290^{b}	79.50±0.500^{ab}	81.50±0.500^{ab}	84.00±1.410^{a}
ABN (%)	1.25±0.750^{a}	0.50±0.288^{b}	2.00±0.707^{a}	0.10±0.011^{b}	0.25±0.250^{b}

^{a,b,c} values within row with different superscripts differ significantly (P<0.05), MOT: Motility, ACI: Acrosome integrity, MI: Membrane integrity, ABN: Abnormality, PSM: Pumpkin seed milk

Table 2: Means (±SEM) Seminal oxidative stress parameters of WAD buck semen preserved with PSM extender supplemented with Vitamin C during cold storage.

Parameters	Inclusion levels of Vitamin C in PSM extender				
	0mM	2mM	4mM	6mM	8mM
MDA (n/mol)	0.23±0.001^{a}	0.06±0.003^{b}	0.03±0.006^{c}	0.04±0.008^{c}	0.01±0.000^{d}
ARG (Unit/mgProtein)	0.82±0.007^{e}	1.54±0.000^{d}	1.56±0.000^{c}	1.82±0.000^{a}	1.58±0.000^{b}
ACR (µlUsperm/106)	0.14±0.002^{d}	0.46±0.022^{d}	1.70±0.059^{c}	3.89±0.433^{b}	6.75±0.125^{a}

^{a,b,c} values within row with different superscripts differ significantly (P<0.05), MOT: Motility, ACI: Acrosome integrity, MI: Membrane integrity, ABN: Abnormality, PSM: Pumpkin seed milk

Table 3: Means (±SEM) *in vitro* capacitation (%) and acrosome reaction (%) of WAD buck spermatozoa preserved with PSM extender supplemented with Vitamin C during cold storage.

Parameters	Inclusion levels of Vitamin C in PSM extender				
	0mM	2mM	4mM	6mM	8mM
In vitro Sperm Capacitation (%)	18.75±2.625^{c}	28.00±4.320^{b}	32.00±6.733^{b}	33.00±6.806^{b}	44.00±6.733^{a}
In vitro Acrosome Reaction (%)	42.00±2.5820^{c}	59.00±9.4340^{abc}	51.00±10.6301^{c}	81.00±5.7446^{a}	74.00±7.7460^{ab}

^{a,b,c} values within rows with different superscripts differ significantly (P<0.05), PSM: Pumpkin seed milk

Result

Table 1 shows the effects of inclusion levels of Vitamin C in 20% PSM extender on sperm viability parameters. The results showed a linear increment pattern for motility, acrosome and membrane integrity parameters with increasing inclusion levels of Vitamin C. Higher (P<0.05) percentage motility was observed at 4mM, 6mM and 8mM inclusion levels of Vitamin C. Membrane integrity also showed similar trend although optimal percentage membrane integrity was observed at 8mM Vitamin C level and higher (P<0.05) acrosome integrity obtained in all levels of supplementations of Vitamin C compared to the control. Inclusions of 2mM, 6mM and 8mM vitamin C in Tris PSM based extenders lowers significantly (P<0.05) sperm abnormality compared to 0mM and 4mM vitamin C supplementation.

Result of oxidative stress parameters of WAD buck spermatozoa during cold storage is presented in Table 2. Reduced (P<0.05) concentration of malondialdehyde was observed at 8mM inclusion levels of Vitamin C compared to other levels of vitamin C and the control. Highest (P<0.05) arginase activity values and acrosin activity values were observed in PSM extender supplemented with 6mM and 8mM levels of Vitamin C respectively.

Table 3 showed the sperm fertilizing parameters of WAD buck spermatozoa during

cold storage with varying inclusion levels of Vitamin C in PSM extender. Higher (P<0.05) percentage sperm capacitation was observed at 8mM of Vitamin C supplementation in PSM extender, while higher (P<0.05) percentage *in vitro* acrosome reaction was observed at 6mM and 8mM.

Discussion

Sustainability potentials of Tris PSM extender supplemented with Vitamin C on sperm motility, and acrosome and membrane integrity.

Addition of Vitamin C to PSM extender in this study sustained the percentage of sperm motility. This observation further show the beneficial effects of Vitamin C on sperm motility and this is due to its role as antioxidant to scavenge free radicals. Vitamin C has been reported to act as an oxidant at low concentrations and as an antioxidant at high concentrations (Affranchino *et al.,* 1991; Breininger *et al.,* 2005). Beconi et al. (1993) reported Vitamin C at a concentration of 5mM, act as an antioxidant in freezing diluents for bovine semen. The present study showed that 4mM to 8mM level of Vitamin C, motility of stored spermatozoa during cold (5oC) storage was maintained. The importance of antioxidants and its effectiveness in many biological systems is invaluable (Pierre *et al.,* 1994; Kazez *et al.,* 2000; Lee *et al.,* 2001). Daramola and Adekunle, (2014) reported the beneficial effect of Vitamin C on buck semen during cold storage. In the present study, the sustainability potentials of PSM extender supplemented with vitamin C on acrosome and membrane integrity of spermatozoa could be as a result of the potent ability of vitamin C to scavenge free radical and or the endogenous phenolic compounds in PSM one of which is Vitamin E (USDA 2016), which are able to scavenge superoxide anions and singlet oxygen thereby protecting lipoproteins of sperm cells from detectable peroxidative damage (Wainer *et al.,* 1986, Donnelly *et al.,* 1999). In contrast Aurich et al. (1997) reported that addition of vitamin C did not improve the motility of cooled equine spermatozoa during the 96-hour storage period but reported increased maintenance of intact spermatozoa membrane. However, Daramola and Adekunle (2015), clarified that the wholesome effects of fruit juice or extract in semen extender on sperm viability cannot only be due to effects of vitamin C alone but such optimal effects could arise from the synergistic effects of other phenolic compounds in the fruit. Moreover, the milk of pumpkin seed contains oil that is rich in unsaturated fatty acids and phytochemicals (Andjelkovic *et al.,* 2010; Rezig *et al.,* 2012), which could have help to confer membrane stability in the stored spermatozoa. This could be on the account that this oil which is also a source of lecithin may have played a protective role during cold storage due to low viscosity, improvement of the kinematics of sperm membrane and rearrangements of phospholipid of the sperm cells membrane as reported by Fukui *et al.,* (2008). PSM is rich in carbohydrates (USDA 2016) which are sources of sugar. Sugar is known to increase the osmostic potential of cells and protect membrane from chilling-induced injury (Purdy, 2006). The lower percentage abnormality obtained at 2 mM, 6 mM, and 8mM, showed that the extenders supplemented with these levels Vitamin C compared to the control after post-chilling suggested that the supplementation had beneficial effects on sperm morphology, this finding is in line with that of Daramola and Adekunle, (2015) that vitamin C and other endogenous ingredients of fruit juices or extracts had beneficial effects on sperm morphology during cold storage of WAD goat buck spermatozoa. Furthermore, semen processing has been reported to have minimal influence sperm abnormality (Revell, 2003).

Protective effects of Tris PSM extender supplemented with vitamin C against Oxidative Stress during cold storage

Malondialdehyde Concentration, as indices of lipid peroxidation was observed to be reduced with increasing levels of Vitamin C during *in vitro* study. This observation further substantiate the previous report of (Affranchino *et al.,* 1991; Breininger *et al.,* 2005; Beconi *et al.,* 1993; Daramola and Adekunle,

2014) that vitamin C is a strong phenolic that help to scavenge free radicals which are products of cellular respiration. The ability of the PSM extender supplemented with Vitamin C to reduce MDA concentration could also be as a result of the milk richness in other Vitamins like vitamin B6 which has been reported to be effective in reducing seminal oxidative stress during semen preservation when supplemented in semen extender Daramola et al. (2016). However, a significant negative correlation between seminal ascorbic acid and MDA had been reported and indicates the defensive role of ascorbic acid against the lipid peroxidation process Piyali *et al.,* (2009).

In the present study, elevated arginase activity indicated the effectiveness of Vitamin C supplementation in PSM extender. Increased arginase activity will consequently reduce or inhibit nitric oxide synthase activity, therefore inhibiting excessive nitric oxide (NO) production (Aydogdu *et al.,* 2006). During stress a negative correlation was reported between NO concentration and, the percentage of rapid progressive motility and arginase activity (Eskiocak *et al.,* 2006), positive correlation was also reported between sperm motility and arginase activity in seminal plasma and spermatozoa (Elgun *et al.,* 2000; Gür and Kandemir, 2012). However, this nitrogen derived free radical nitric oxide, also appear to play a significant role in reproduction and fertilization Rosselli *et al.,* (1998), over production of the free radical and the consequent excessive exposure to oxidative conditions have a potential pathogenetic implication in the reduction of sperm motility (Garg and Garg 2011). PSM extender is rich in antioxidants, which is capable of reducing superoxide radical and lipid peroxide levels induced by H2O2 in the vascular endothelial cells (Mahfouz *et al.,* 2009). The elevated arginase activity of the present study indicating that Vitamin C in PSM extender helps to increase or maintain the arginase activity of stored WAD buck semen consequently maintaining motility and viability, this corroborates the work of Elgun et al. (2000) that increased arginase activity generally results in lower NO concentration and subsequently increased sperm motility. In mammalian sperm, the presence of acrosin was highly correlated with sperm penetration of *in vitro* mature oocytes and zona pellucida, and this has been proposed to be an indicator for sperm quality (Rosatti *et al.,* 2003). Estradal *et al.,* (2015) in his work reported that sperm freezing significantly reduce acrosin activity in mammals, and that the loss of acrosome integrity underlie the decrease in acrosin activity. The present study, which is a cold storage of WAD buck sperm at 5°C using Tris PSM extender supplemented with vitamin C, revealed sustenance of acrosomal integrity which could have also resulted in the retention of the enzyme. These findings could also be attributed to the supplementation of vitamin C in PSM extender which helps to better maintain acrosin activity corroborating the findings of Paudel *et al.,* (2010) that addition of ascorbic acid, to preservation media not only reduce sperm storage damage in bulls but also maintain their acrosin activity.

Effectiveness of Vitamin C in PSM extender in maintaining sperm fertilizing parameters during cold storage.

Mammalian spermatozoa undergo capacitation, a series of intracellular and membrane physicochemical changes that give spermatozoa ability to fertilize ova (Patrat *et al.,* 2000). Only capacitated spermatozoa have ability to undergo acrosome reaction (Arnoult *et al.,* 1996). Evaluation of acrosome reaction can be used to predict success of fertilization in artificial insemination programme. PSM is compact with vitamins such as (vitamins A, B, C or E) and minerals such as sodium and potassium (USDA 2016). In the present study, supplementation of vitamin C into PSM extender and the endogenous compounds of PSM could have resulted into the present observations in which Vitamin C in PSM extender maintained the fertilizing capacity (sperm capacitation and acrosmen reaction) of goat sperm after cold storage for 96 hours. This result is supported by the work of Daramola *et al.,* (2015) who reported the beneficial effects of pyridoxine in combination with antioxidants such as vitamin C on sperm motility, capacitation and acrosome

reaction during preservation. In addition, the results were in consonance with El-Nasry *et al.,* (2004) who found that the supplementation of some antioxidants (vitamins A, C or E) to semen extenders resulted in significant improvement in motility, viability, morphology and fertilizing ability of roosters semen after *in vitro* storage. Furthermore, potassium and sodium present in the PSM could have also contributed their own protective properties. Sperm viability was reported to be influenced by the potassium levels in storage medium and the beneficial effect of potassium on the viability of diluted spermatozoa has been demonstrated (Mansour *et al.,* 2002). Sodium is an organic compound which apparently cannot cross the cell membranes but possesses protective properties against cold exposure (Toner *et al.,* 1993) helps to maintain sperm viability during storage. All these attribute of PSM and the higher supplementation levels of Vitamin C in PSM extender maintains the viability of goat sperm to undergo *in vitro* capacitation and acrosome reactions consequently maintaining the fertilizing integrity of goat sperm preserved for 96 hours at 5°C storage.

Conclusion

The findings of the study revealed that addition of Vitamin C at 6mM and 8mM levels to PSM extender maintained consistently the viability parameters of goat buck spermatozoa during cold storage at 5°C. Combination of vitamin C with pumpkin seed milk shows a promising diluent for goat semen and the prospect of this can be utilised in artificial insemination programmes. However, further studies needs to be done on PSM extender and vitamin C on conception rate, non-return to estrus and its potentials as semen diluent in other semen preservation methods.

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SEROEPIDEMIOLOGY OF CONTAGIOUS BOVINE PLEUROPNEUMONIA (CBPP) IN KATSINA STATE, NIGERIA

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Abstract

A cross sectional study was carried out on 200 cattle from 108 purposively selected herds in Mai'adua, Dutsinma and Funtua Local Government Areas representing Northern, Central and Southern Districts of Katsina state to determine herd and animal level seroprevalence of CBPP and to also identify risk factors of transmission of the disease in the state. Latex Agglutination Test (LAT) kit was used to assay for antibodies against *Mycoplasma mycoides mycoides* Small Colony (MmmSC). Structured questionnaires were used to generate data on possible risk factors to CBPP in the state. Herd level and animal level CBPP seroprevalence of 61.1% with odds ratio of 0.8 (95 % confidence interval (CI) = 0.34-1.73) and 47.5% with odds ratio of 1.8 (95% CI = 0.52-6.39) respectively was found. It was concluded that persistence of opportunities for contact between infected herds and or individual animals with susceptible ones will continue to sustain transmission and prevalence of CBPP in Katsina state. The Katsina state government should review its control strategy on CBPP and develop more intensified initiatives that will reach the ordinary herdsman in the rural community. Control, elimination and final eradication measures should be developed and well funded towards this disease of economic importance.

Keywords: CBPP, Cattle, Seroprevalence, Mai'adua, Dutsinma, Katsina

SEROEPIDEMIOLOGIE DE LA PLEUROPNEUMONIE CONTAGIEUSE BOVINE (CBPP) DANS L'ETAT DE KATSINA AU NIGERIA

Resume

Une étude transversale a été réalisée sur 200 bovins provenant de 108 troupeaux choisis à dessein dans les districts de May'adua, Dutsinma et Funtua représentant les districts nord, centre et sud de l'État de Katsina pour déterminer la séroprévalence de la PPCB au niveau du troupeau et de l'animal et définir les facteurs de risque de transmission de la maladie dans cet État. Le kit du Test de l'agglutination au latex (LAT) a été utilisé pour tester les anticorps contre Mycoplasma mycoides mycoides « Small Colony » (MmmSC). Des questionnaires structurés ont été utilisés pour générer des données sur les facteurs de risque possibles de la péripneumonie contagieuse bovine dans cet État. La séroprévalence de la PPCB au niveau du troupeau et de l'animal était de 61,1%, respectivement avec un rapport de cotes de 0,8 (intervalle de confiance à 95% = 0,34-1,73) et de 47,5% avec un rapport de cotes de 1,8 (IC à 95% = 0,52-6,39). Il a été conclu que la persistance des possibilités de contact entre les troupeaux infectés et / ou les individus et les animaux sensibles continuerait à maintenir la transmission et la prévalence de la péripneumonie contagieuse bovine dans l'État de Katsina. Le gouvernement de l'État de Katsina devrait revoir sa stratégie de contrôle de la péripneumonie contagieuse bovine et développer des initiatives plus intensives susceptibles d'atteindre le berger ordinaire dans la communauté rurale. Des mesures de contrôle, d'élimination et d'éradication finale devraient être développées et bien financées afin de lutter contre cette maladie d'importance économique.

Mots-clés : PPCB, bovins, séroprévalence, Mai'adua, Dutsinma, Katsina

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Introduction

Contagious bovine pleuropneumonia (CBPP) caused by *Mycoplasma mycoides* subspecies *mycoides* Small Colony (MmmSC) variant, is an important trans-boundary animal disease, particularly in Africa (Mtui-Malamsha, 2009). CBPP is a major obstacle to cattle production in Africa and is regarded as the most important infectious disease of cattle especially with the recent eradication of rinderpest from the continent (Mariner *et al.,* 2006). Annual losses of up to thirty million euros (€30 Million) due to CBPP were estimated in 12 endemic sub-Saharan African countries (Tambi *et al.,* 2006).

In Nigeria the true situation of CBPP prevalence is not well established but likely increasing on a daily basis due to inadequate funding of cattle annual mass vaccination programs, lack of a rapid on farm screening test to aid sero-monitoring, and refusal of some farmers to allow vaccination of their animals (Amanfu, 2009). Rapid increase in the herd sero-positivity as previously reported indicated alarming increase in the prevalence of the disease (Danbirni *et al.,* 2010; Suleiman *et al.,* 2015). In Katsina state of Nigeria, an overall infection rate of 16.7% in adults and 17.5% in calves out of 120 animals screened for the disease indicates a low infection rates for a disease that is transmitted through contact, ingestion and aerosol (Okaiyeto *et al.,* 2011). Field based evidence for CBPP prevalence is the pillar upon which efforts on control activities depends (Thomson, 2005). The study was designed and conducted to answer the null hypothesis statement that there is no significant prevalence of CBPP in Katsina State, Nigeria. The objectives of the study was to determine herd and animal level seroprevalence of CBPP and to also identify risk factors of transmission of the disease in the state.

Materials and Method

The study was conducted in Katsina state which occupies an area of 23,938 sq. km and is located between latitudes 11°08'N and 13°22'N and longitudes 6°52'E and 9°20'E. The state is bordered by Niger Republic to the north, by Jigawa and Kano States to the east, by Kaduna State to the South and by Zamfara State to the West. Katsina State forms part of the extensive plains known as the High Plains of Hausa land (Isa, 2009).

A cross sectional survey was carried out in three (3) LGAs of Katsina state (Mai'adua, Dutsin-Ma and Funtua). Serological examination was also carried out on the blood samples collected to determine animal and herd level CBPP seroprevalence. Face to Face interviews were used to serve the structured questionnaires that were used to assess the level of awareness and to identify risk factors to the transmission of the disease.

A sample size of 200 was estimated from an expected seroprevalence of 31% (Suleiman *et al.,* 2015) as described by Thrusfield (2007). One herd man was interviewed per herd visited and both verbal and visual observations were used to validate response.

Herds were sampled based on the herdsmen consent, while cattle within the herds were bled for serum. Numbers of herds sampled per selected LGA were based on the willingness of the herdsmen and hence social mobilization meetings, use of attractive pluses (dewormers, and oral appetite stimulants) and sensitizations were carried out before sampling. Sampling units comprised of single herds and 10% of animals in each herd were bled.

The serum samples were analyzed using the Latex Agglutination Test (LAT kit) as described by described by March et al (2003).

Animal and Herd level seroprevalence was calculated with the formula:

$$\text{Prevalence (\%)} = \frac{\text{Number of animals that test positive to LAT}}{\text{Total number of animals sampled during the study period}} \times 100$$

Herd level seroprevalence was calculated using the formula below:

$$\text{Prevalence (\%)} = \frac{\text{Number of herds that have at least one animal positive to LAT}}{\text{Total number of herds sampled during the study period}} \times 100$$

Inferential statistics using Odds ratio (OR) and 95% confidence interval (CI) on the Odds ratio were calculated using Winepiscope® 2.0 to measure strengths of statistical significance of associations between variables and CBPP serostatus. Values of $p \leq 0.05$ were considered significant. Descriptive statistics was carried out using Microsoft excel® 2007.

Results and Discussion

In this study an overall herd level prevalence of 61.1% (95 % confidence interval (CI) = 0.34-1.73) out of 108 herds screened indicates probably a high infection rates for a disease that is transmitted through contact, ingestion and aerosol. This report is not in variance with that of Okaiyeto *et al.,* (2011) where they reported a prevalence of 17.5% using similar diagnostic kit in an on farm diagnosis of CBPP in Kafur LGA, Katsina state; and that of McDermott *et al.,*(1987) and Dasho (2001) where they also reported serological values of 8.1 to 9.2% prevalence in southern Sudan and Ethiopia. However, the high herd level prevalence of this study agreed with that of Suleiman *et al.,* (2015) where they reported a herd level prevalence of CBPP of 54.7 % (95 % confidence interval (CI) = 47.7–62.0) using compliment Enzyme Linked Immunosorbent Assay in a similar study conducted in Kaduna state Nigeria.

The present study established that CBPP seroprevalence was significantly associated with risk factors describing aspects of livestock contacts and herds dynamics. Herd exposure to other animals have been reported to determine CBPP distribution patterns in many African pastoral systems Mariner *et al.,* (2006) and risk factors for the disease spread were shown to mainly include high-density confinement in night housings and use of common pastures and watering places Provost *et al.,* (1987). Congregation of cattle at pastures and or watering points is one of the major determinants of CBPP seropositive herds in Katsina state. This maybe particularly evident during dry seasons when grazing and water sources are limited. Such exposures may lead to CBPP transmission between infected and susceptible herds Suleiman *et al.,* (2015).

Table 1: Animal Level Seroprevalence of Cattle Using LAT According to Sampled LGAs

LGA	No. Screened	No. Positive	Prevalence (%)	OR	95% CI on OR
Mai'adua	46	30	65.2	1.8	0.52-6.39*
Dutsinma	83	41	49.4	3.9	1.41-10.84*
Funtua	71	24	33.8	0.3	0.12-0.95*
Total	200	95	47.5		

**Significant at 95%CI*

Table 2: Herd Level Seroprevalence of CBPP in Katsina state

Herd size	Herds screened	Positive herds	Prevalence (%)	OR	95% CI on OR
Less than 20	24	21	87.5	3.0	0.20-44.36*
20 to 40	45	18	40.0	0.3	0.08-0.99
More than 40	39	27	69.2	1.00	0.23-4.23
Total	108	66	61.1	0.8	

**Significant at 95%CI*

Table 3: Distribution of CBPP herds according to herd dynamics in Katsina state

Variable	Status (Yes/No)	Herds Sampled	Positive Herds	Prevalence (%)	OR	95% CI on OR
Brought Inn	Yes	31	27	87.1	1.9	0.59-6.23
	No	77	60			
Sold Out	Yes	61	38	77.9	0.77	0.35-1.73
	No	47	32			
Gift Inn	Yes	29	18	62.1	0.59	0.24-1.46
	No	79	58			
Brought back from market	Yes	10	7	70	2.24	0.55-9.17*
	No	98	50			
Congregatingat grazing / watering point	Yes	67	48	72	0.43	0.16-1.20*
	No	41	35			

**Significant at 95%CI*

Conclusion

The present study established that CBPP seroprevalence was significantly associated with risk factors describing aspects of livestock contacts and herds dynamics. The study therefore concludes that herds in the state may be at risk of being CBPP seropositive due to increased frequency and duration of contact between infected and susceptible animals.

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Conflict of Interests

The authors wish to declare that there is no conflict of interest to this submission.

Public Brief on the Study for the Benefit of Policy Makers

Due to the high prevalence of this disease as shown in this study, The Katsina state government should review its control strategy on CBPP and develop more intensified initiatives that will reach the ordinary herdsman in the rural community. Control, elimination and final eradication measures should be developed and well funded towards this disease of economic importance.

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PERFORMANCE CHARACTERISTICS AND APPARENT NUTRIENT DIGESTIBILITY OF BROILER CHICKENS FED DIETS SUPPLEMENTED WITH PHYTO-ADDITIVES

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Abstract

A 56 d study was conducted to determine the effects of dietary inclusion of *Moringa* (*Moringa oleifera*) leafmeal, Neem (*Azadirachta indica*) leaf meal and Bitter leaf (*Vernonia amygdalina*) meal on the growth performance and apparent nutrient digestibility of broiler chickens. Four hundred (400) 1- d old unsexed Ross 308 broiler chickens were randomly allotted to five diets consisting either a basal diet without supplement (negative control) or basal diet with 5mg/kg commercial antibiotic (positive control) or basal diet with 5mg/kg phytobiotics (moringa leaf meal (MLM),neem leaf meal (NLM) and bitter leaf meal (BLM) for 1-56 d. Each dietary treatment consisted of 4 replicates of 20 birds with a replicate being an experimental unit. The phyto-additives were assayed for chemical compositions. The phytochemical screening revealed the presence of tannin, alkaloids, flavonoid, Phenol and Saponin. Birds fed diets supplemented with commercial antibiotic and MLM were heavier ($p<0.05$) while those on control and NLM had poorest weight at 28 d and 56 d. MLM and BLM diets were more consumed ($p<0.05$) by the starter birds. However, feed consumption was not influenced by the diets at the finisher phase. Birds on phyto-additives had higher percentage ($p<0.05$) of survival compared to those control and antibiotics at 28 and 56 d. Best feed:gain was achieved when the diet was supplemented with commercial antibiotics at 28 d and antibiotic and MLM at 56 d. The digestibility of dry matter (86.96%) and ash (75.34%) were higher ($p<0.05$) in birds given feed containing antibiotic improved ($P<0.05$) digestibility of crude protein and ether was observed in Birds fed diet containing commercial antibiotics.

Keywords: Broiler chickens, phyto-additives, and performance characteristic

CARACTERISTIQUES DE PERFORMANCE ET DIGESTIBILITE APPARENTE DES NUTRIMENTS DES POULETS DE CHAIR SUPPLEMENTES EN PHYTO-ADDITIFS

Resume

Une étude de 56 jours a été réalisée dans le but de déterminer les effets de l'inclusion alimentaire de la farine de feuilles de Moringa (Moringa oleifera), de la farine de feuilles de Neem (Azadirachta indica) et de la feuille amère (Vernonia amygdalina) sur la croissance et la digestibilité apparente des poulets de chair. Quatre cents (400) poulets de chair Ross 308s des deux sexes, âgés d'un (1) jour, ont été aléatoirement répartis à cinq régimes comprenant soit un régime de base sans supplément (témoin négatif), soit un régime de base avec 5 mg / kg d'antibiotique commercial (témoin positif) ou un régime de base avec 5 mg / kg de phytobiotiques (farine de feuilles de moringa - MLM, farine de feuilles de neem –NLM- et farine de feuilles amères -BLM) pendant 1 à 56 jours. Chaque traitement diététique consistait en 4 répétitions de 20 oiseaux, une répétition étant une unité expérimentale. Les phyto-additifs ont été étudiés pour déterminer leurs compositions chimiques. Le dépistage phytochimique a révélé la présence de tanins, d'alcaloïdes, de flavonoïdes, de phénol et de saponine. Les oiseaux recevant des suppléments d'antibiotiques commerciaux et de MLM étaient plus lourds ($p <0,05$) que ceux soumis au régime témoin, et ceux recevant NLM avaient le poids le plus faible aux jours 28 et 56. Les régimes MLM et BLM étaient plus consommés ($p <0,05$) par les jeunes oiseaux. Cependant, la consommation d'aliments n'a pas été influencée par les régimes en phase de finition. Les oiseaux recevant des phyto-additifs avaient un pourcentage de survie plus élevé ($p <0,05$)

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que les témoins et les antibiotiques à 28 et 56 jours. Meilleur aliment : gain obtenu lorsque le régime était complété avec des antibiotiques commerciaux au jour 28 et des antibiotiques au jour 56. La digestibilité de la matière sèche (86,96%) et des cendres (75,34%) était plus élevée (p <0,05) chez les oiseaux recevant des aliments contenant des antibiotiques améliorés (P <0,05), la digestibilité de la protéine brute et de l'éther a été observée chez les oiseaux recevant des antibiotiques commerciaux.

Mots-clés : poulets de chair, phyto-additifs, et caractéristiques de performance

Introduction

Antibiotics have been widely used in animal production for decades. Although some are used therapeutically to improve the health and well-being of animals, most were given for prophylactic purposes and as antimicrobial growth promoters (AGPs) (Gerard *et al.,* 2011). The beneficial effects of antibiotics in combating bacterial problems and as growth promoters are well documented (Gerard *et al.,* 2011). However, problems associated with usage of antibiotics are drugs toxicity, residual effects and development of microbial resistance. The use of organic supplements such as probiotics and herbs, are generally believed to be safer, healthier, and less subject to hazards. Thus, herbs and herbal products are incorporated in livestock feeds and water instead of synthetic products in order to stimulate or promote effective use of feed nutrients, which result in more rapid gain, higher production and better feed efficiency (Ghazalah and Ali, 2008). Moreover, herbs contain active substances that can improve digestion and metabolism and possess antibacterial and immune stimulant activities (Ghazalah and Ali, 2008). They are also reported to contain aromatic properties that have impact on gut micro-flora, nutrient digestibility, intestinal morphology and meat quality of poultry (Cross *et al.,* 2010). The medicinal effects of these plant moringa (*Moringa oleifera*), Neem tree (*Azadirachta indica*) and bitter leaf (*Vernonia amygdalina*) was ascribed to their possession of anti-oxidants, which are known to suppress the formation of reactive oxygen species (ROS) and free radicals (Sofidiya *et al.,* 2006; Ogbunugafor *et al.,* 2011), based on this effects, this study was designed to determine Performance characteristics, apparent nutrient utilization and carcass characteristics of broiler chickens fed diets supplemented with phyto-additives which include Moringa leaf meal (MLM), Neem leaf meal (NLM) and Bitter leaf meal (BLM)

Materials and Methods

This study was carried out at the Poultry unit of the University Farms, Federal University of Agriculture, Abeokuta, Alabata Road, Nigeria. Alabata is in Odeda Local Government Area of Ogun State. It falls within the rainforest vegetation zone of the south-west Nigeria on the latitude 7°S 13' 49.66'N and longitude 3°26'11.98 E and 76 metres above sea level (Google earth, 2017). The climate is tropical humid with a mean annual rainfall of 1037mm

Processing of test ingredients

The fresh, green and undamaged mature leaves of *Moringa oleifera*, *Azadirachta indica* and *Vernonia amygdalina* were harvested from a variety of trees in the same environment. The leaves were air-dried during the day without direct sunlight exposure for 5 days until they were crispy to touch. The leaves were milled to pass through 2 mm sieve to obtain *Moringa* leaf meal (MLM), Neem leaf meal (NLM) and Bitter leaf meal (BLM). The analysed nutrient and phytochemical composition of the leaves are shown in Table 2.

Experimental design

400 day-old unsexed Ross 308 broiler chicks were randomly assigned to 5 dietary groups in a Completely Randomized Design (CRD). Each dietary group consisted of 80 birds with 4 replicates of 20 birds each. The study was conducted for two phases of starting and finishing of 28 days each.

Dietary Treatment

A corn-soybean based diet was formulated to meet the nutrient requirement of birds (NRC, 1994) for the starter broilers (0 to 28 d) and finisher broilers (29 to 56 d). Five dietary treatment groups were produced from a basal feed such that: diet 1 was negative control (without supplementation), diet 2 was positive control with 5g/kg commercial antibiotic (Keproceryl® WSP containing Colistin 22,5000 IU, Oxytetracyclin 50 mg, Erythromycin 35 mg and Streptomycin 35 mg), diets 3, 4, 5 were supplemented with 5g/kg MLM, NLM and BLM respectively. The ingredients and diets composition are presented in Table 1.

Birds and Management

A total of 400 d-old unsexed Ross 308 broiler chicks were purchased from AGRITED, a commercial hatchery in Ibadan. The birds were weighed and reared in 20 floor pens (each measuring 2.0m x 1.5m) containing fresh wood shavings. Each unit housed 20 birds. Brooding was done for 21days at 36°C pen temperature for the first one week and then reduced by 3°C per week until 27°C was reached and maintained during the remaining rearing period. The birds did not receive any vaccination other than Marek's and Newcastle disease vaccine received at Hatchery. Feed and fresh water were supplied ad libitum throughout the rearing period.

Chemical analysis

The nutrient and phytochemical composition of the ground samples (n=4) of the dried leaves of *Moringa oleifera*, *Azadirachta indica* and *Vernonia amygdalina* Table 2, feed and faecal samples were determined according to the standard procedures of AOAC (1995). The nitrogen fraction of the feed samples was determined using Kjeldahl distillation method and crude protein (CP) was determined by multiplying the N value by 6.25. Tannin was determined by the Folin-Dennis spectrophotometric method as described by Makka (1989).Alkaloids and cyanogenic glucoside were determined by the gravimetric precipitation method as described by Harbone (1973). Flavonoid was determined following the method of Boham and Kocipai –Abyazan (1994). Saponin was determined by method described by Obadoni and Ochuko (2002).

Measurement of apparent nutrient digestibility

At 56 d three birds per pen (making a total of 12 birds per treatment) were randomly selected and housed individually in metabolic cages. A 3-day acclimatization period was allowed prior to a 4-day collection period. Birds were fed with the quantity of feed which exceeded their daily feed intake. Daily excreta voided per bird were collected and dried at 65°C in an air-draft oven to a constant weight. Excreta obtained from each replicate were pooled together and ground to pass through 1 mm screen. Ground feed and faecal samples were used to determine their respective proximate constituents (AOAC 1995).

Data collection

Data on growth indices such as body weight, weight gain feed intake and feed conversion ratio were collected weekly.

Statistical Analysis

Data collected were subjected to one-way analysis of variance in a Complete Randomized Design using SAS (2000) while significance means separated according to Tukey's test and were considered to be significant when $P<0.05$.

Results

The result of the growth performance is presented in Table 2. The final weight, weight gain, feed intake and Feed: Gain were significantly ($P< 0.05$) influenced by dietary treatments at starter and finisher phases. The final weight and weight gain of broilers fed diets supplemented with commercial antibiotics and MLM were higher compared to those on control and other diets at both starter and finisher phases. Broilers on NLM diet consumed significantly less feed, while the intake of birds on other treatments were similar ($P>0.05$) at both phases. The Feed: Gain recorded for birds fed diets supplemented

Table 1: Composition of basal starter and finisher diets (g/kg)

Ingredients	Starter (0-28 d)	Finisher (29-56 d)
Maize	480.0	580.0
Soybean	220.0	150.0
Ground nut cake	120.0	140.0
Fish meal (72% CP)	20.0	20.0
Wheat offal	100.0	-
Bone meal	20.0	20.0
Oyster shell	30.0	30.0
*Vitamin + Mineral Premix	2.5	2.5
Salt (NaCl)	2.5	2.5
Lysine	2.0	2.0
Methionine	3.0	3.0
Total	1000	1000
Analysed Composition (g/kg)		
Crude protein	221.6	201.2
Ether extract	38.4	39.9
Crude fibre	36.6	30.6
Calcium	17.5	17.4
Available phosphorus	4.3	4.2
Calculated Composition		
+ME (MJ/kg)	11.68	12.1

**2..5 kg Premix supply the following per ton of feed: Vitamin A 12,000,000 I U;Vitamin D3 3,000,000 I.U;Vitamin E 30,000,000;Vitamin K 2,500mg;folic acid 1,000mg;Niacin 40,000mg;pantholelic acid 10,000mg;Vitamin B12 20mg;Vitamin B 12,000mg;Vitamin B6 3,500mg;Biotin 80mg;Antioxidant 125,000;Colbact 250mg;Selenium 250mg;iodine 200mg, Iron 40,000mg; Manganese 70,000mg;Copper 8,000mg,Zinc 60,000mg;chlorine 200,000mg. + = 5g/kg, MLM=Moringa oleifera leaf meal, NLM=Neem leaf meal; BLM=Bitter leaf meal*
+ME (MJ/kg) = (37 x %CP + 81.1 x %EE + 35.5 x %NFE) x 4.18/1000 (Pauzenga, 1985)

Table 2: Analysed nutrient and phytochemical composition of Moringa Leaf Meal (MLM), Neem Leaf Meal (NLM) and Bitter Leaf Meal (BLM)

Parameters	MLM	NLM	BLM
Nutritional composition (g/100g)			
Dry matter	90.55	92.56	90.82
Crude protein	25.89	18.01	18.48
Ether extract	4.78	3.00	7.81
Crude fibre	12.55	15.38	14.86
Ash	9.23	9.97	4.55
Nitrogen free extract	39.10	46.20	45.12
*Metabolizable energy (MJ/kg)	11.43	10.66	12.26
Phytochemical composition (mg/g)			
Tannin	45.0	12.5	95.0
Flavonoid	24.7	16.3	39.5
Alkaloids	85.0	27.4	31.5
Phenol	59.0	37.0	27.0
Saponin	74.0	13.8	73.0

**Metabolizable energy (MJ/kg) = (37 x %CP + 81.1 x %EE + 35.5 x %NFE) x 4.18/1000 (Pauzenga, 1985)*

Table 3: Effects of diets supplemented with or without phyto-additives on growth performance of broiler chickens

Parameters (%)	Control	Antibiotics	MLM	NLM	BLM
Starter Broilers (28 d)					
Average initial weight (g)	43.87±0.42	43.87±0.42	44.00±0.46	44.00±0.46	44.00±0.46
Average Final weight (g)	737.07±42.23^{bc}	1129.00±28.97^{a}	1020.82±35.61^{a}	738.50±21.02^{bc}	855.5±47.5b
Average weight gain/bird(g)	693.20±42.26^{c}	1085.29±29.13^{a}	976.83±35.48^{a}	694.50±20.70^{c}	811.90±47.53^{b}
Total feed intake/b(g)	1854.50±34.9^{a}	1962.25±89.20^{a}	2110.25±19.20^{a}	1551.50±83.97^{b}	2092.00±150.75^{a}
Average feed intake/b/d (g)	66.23±1.24^{b}	70.08±3.18^{b}	75.37±0.68	55.41±2.99^{c}	74.71±5.38^{a}
Feed: Gain	2.69±0.11^{a}	1.82±0.12^{c}	2.16±0.19^{b}	2.23±0.12^{ab}	2.60±0.24^{a}
Livability (%)	85.00±0.12^{c}	90.00±0.12^{b}	95.00±0.24^{a}	95.00±0.11^{a}	95.00±0.19^{a}
Finisher Broilers (56 d)					
Average initial weight (g)	737.07±42.23^{bc}	1129.00±28.97^{a}	1020.82±35.61^{a}	738.50±21.02^{bc}	855.5±47.5^{b}
Average Final weight (g)	2397.25±125.93^{b}	3103.75±157.24^{a}	2799.00±157.24^{ab}	1898.25±235.05^{c}	2217.25±75.69^{bc}
Average weight gain/bird(g)	1376.42±118.69^{b}	1974.75±177.34^{a}	1778.18±162.30^{ab}	1159.75±251.89^{c}	1361.32±122.50^{b}
Total feed intake/b(g)	4670.00±98.83^{a}	4355.00±376.00^{a}	3842.00±255.5^{ab}	3020.00±343.27^{a}	4681.50±200.00^{b}
Average feed intake/b/d (g)	166.79±3.5^{a}	155.54±13.00^{a}	137.24±9.00^{ab}	107.85±12.2^{b}	167.20±7.10^{a}
Feed:Gain	2.68±0.22^{ab}	2.29±0.35^{b}	2.16±0.24^{b}	2.78±0.35^{ab}	3.51±0.28^{a}
Livability (%)	85.00±0.24^{c}	90.00±0.28^{b}	99.00±0.22^{a}	99.00±0.35^{a}	99.00±0.22^{a}

^{abc}*Means in the same row having different superscripts are significantly different (P<0.05)*

Table 4: Effects of diets supplemented with or without phyto-additives on nutrient digestibility of broiler chickens

Parameters (%)	Control	Antibiotics	MLM	NLM	BLM
Starter Broilers (28 d)					
Dry Matter Digestibility	78.23±0.40^{c}	86.96±0.54^{a}	83.33±0.45^{b}	70.66±0.35^{d}	63.34±1.03^{e}
Crude Protein Retention	77.59±0.29	80.31±0.35	88.47±0.67	62.65±0.29	64.39±0.74
Ether Extract Digestibility	78.87±0.51	88.52±0.29	77.27±0.75	63.23±1.04	65.03±1.48
Crude Fibre digestibility	70.49±0.45^{b}	65.64±0.57^{c}	73.48±1.06^{a}	69.80±0.44^{b}	68.12±0.38^{bc}
Ash Digestibility	68.15±0.49^{b}	75.34±1.30^{a}	71.03±0.50^{b}	56.60±0.51^{c}	69.93±0.53^{b}
Finisher Broilers (56 d)					
Dry Matter Digestibility	73.24±1.07^{b}	80.55±0.23^{a}	75.64±0.23^{b}	66.05±0.54^{c}	71.52±0.29^{b}
Crude Protein Retention	72.55±0.36^{b}	81.01±1.43^{a}	71.1±0.65^{b}	65.39±0.51^{c}	71.01±1.49^{b}
Ether Extract Digestibility	63.71±0.83^{c}	74.30±1.19^{a}	66.98±0.55^{b}	67.67±0.97^{b}	64.43±0.44^{c}
Crude Fibre digestibility	71.91±0.51^{a}	65.35±0.32^{c}	66.24±1.23^{c}	72.59±1.02^{a}	69.87±0.54^{b}
Ash Digestibility	74.45±1.14^{a}	68.35±0.31^{b}	66.12±2.13bc	67.98±0.73^{b}	63.30±1.11^{c}

^{abce}*Means on the same row having different superscripts were significantly different (P<0.05)*

with commercial antibiotics was better than those on other dietary treatments at starter phase but similar with broilers on MLM diet at finisher phase. Birds on phyto-additives had higher (P<0.05) percentage livability than those on control diet and diet containing antibiotic at starter and finisher phases.

The result of apparent digestibility at starter phase is presented in Table 3 shows that the dry matter (DM), crude fibre (CF) and ash digestibility were significantly influenced (P<0.05) by the dietary treatments. The DM and ash digestibility of diets containing commercial antibiotics were better (P<0.05) than those on other treatments, while those on control diets had the least (P<0.05) DM digestibility. The digestibility of CF was highest (P<0.05) in birds fed diets containing MLM. Those fed control diet and diets containing NLM and BLM recorded a similar CF digestibility values. Those fed diets containing antibiotic recorded the least CF digestibility. The crude protein retention (CPR) and ether extract (EE) digestibility were not influenced by (P>0.05) by the dietary treatments. The result of apparent digestibility of the diets by the finisher broilers (Table 4) showed higher (P<0.05) DM and EE digestibility and CPR values for broilers fed diets containing antibiotics. The DM digestibility and CPR values of MLM, BLM and control diets are similar. Birds fed control and NLM diets recorded similar but higher (P<0.05) CF value compared to those on other diets. Birds fed diets containing commercial antibiotics and MLM had the least CF digestibility. Birds on control diet had highest (P<0.05) ash digestibility while those on BLM diet had the least value

Discussion

Supplementation of diets with commercial antibiotic and MLM improved the final weight and weight gain of the broiler chickens. Antibiotic may have exerted a positive influence on the micro-flora resulting in improved feed utilization and growth. The study reported by Ferket (2004) showed that antibiotics control and limit the growth and colonization of a variety of pathogenic and nonpathogenic species of bacteria in broiler chickens and increase biota population in the gut that can lead to a greater efficiency in digestibility and utilization of feed resulting in an enhanced growth and improved feed conversion ratio. MLM had a similar growth effect as antibiotic on the broiler chickens. The nutritional profile of MLM showed that it is rich in protein. *Moringa oleifera* has been reported to contain high quality amino acids of which 10 out of 19 were considered essential (Moyo *et al.,* 2011). Also MLM assayed in this study contained some bioactive chemicals such as phenols, alkaloids, saponin and tannin with antioxidants properties which may impact positively on nutrient digestibility, nutrient absorption, intestinal morphology (Cross *et al.,* 2007) resulting in improved growth. MLM has been reported to have impressive range of medicinal uses, growth promotion, antimicrobial and antioxidant effects (Mbikay *et al.,* 2012). These qualities could have been responsible for improved performance of broilers on MLM supplemented diets similar to those on diets containing antibiotics. The depression in body weight and weight gain in broilers fed NLM diet could be attributed to low intake of the diet both at 28d and 56d. NLM is bitter; this may have influenced the poor palatability of the diet resulting in low intake and depressed growth. Earlier studies by Kumar and D'Mello (1995) and Onyimonyi *et al.,* (2009) reported a depressed growth in broiler chickens fed diets containing neem leaf when compared to those on control diet.

These results confirmed earlier findings by Esonu *et al.,* (2006); Ogbuewu *et al.,* (2010). High value of feed intake recorded at the finisher phase of broiler chicken fed diets supplemented with BLM is contrary to the report of Olobatoke and Oloniruha (2009) who reported a low intake of diets containing BLM. Poor feed: gain observed in birds fed diets supplemented with BLM at starter and finisher phases is an indication that the feed was not effectively utilized despite the increased intake of the feed. This was in disagreement with the report of Huffman *et al.,* (1996) who

reported that supplementation of bitter leaf in the diet enhance the gastro intestinal enzyme (chymotrypsin) production which improve utilization of feed and digestion of sporozites and other intestinal parasite that could cause decrease in feed utilization.

High mortality observed in broiler chickens fed control diets might be due to the fact that the broiler chickens did not receive any much immune boost from the diet to be able to resist possible infection compared with those fed other test diets. However low mortality recorded in broilers on diets containing MLM, NLM and BLM may be attributed to their antimicrobial and antiviral properties and antibiotic metabolites, such as carboxylic acid, 2,4-diacetyl phloroglucinol, and cell wall-degrading enzymes and chitinases reported by Jabeen *et al.,* (2008) may have helped in survivability of the birds.

The improved digestibility of dry matter, crude fibre, ether extract and ash of broilers on antibiotic diets over the birds on leaf meals and control could be attributed to role of antibiotics in suppressing growth of bacteria that compete with the nutrient in the gastrointestinal tract of the birds. Antibiotics are assumed to improve nutrient digestibility by the inhibition of growth and metabolic activities of intestinal bacteria that compete with the host for nutrients (Engberg *et al.,*2000 ; Engberg and Richards 2005). Generally, the suppression of pathogens always reduces the production of bacterial toxins and consumption of nutrients by bacteria (Keeney and Finlay, 2011). Knarreborg *et al.,*(2004) reported that antibiotics improve absorption of nutrients by thinning the intestinal wall and reducing the amount of growth-depressing metabolites produced by gram-positive bacteria. The relatively low digestibility of most nutrients by birds on leaf meals could be as a result of some bioactive compounds in the leaves. The anti-nutritional effects of tannin, flavonoid, alkaloid, phenol and saponin in reducing the digestibility of the nutrients may not be overruled even though the intake of these compounds was low.

Conclusion

Based on the result, the use of moringa leaf meal (MLM) at 5g/kg compared favourably well with the use of commercial antibiotics in terms of growth performance (final weight, feed intake and Feed: Gain) of the broiler chicken. Improved growth performance was achieved with diet containing MLM compared to those on negative control and other leaf meals. Survivability of the birds was higher in groups fed diets containing leaf meals. With the recent ban of in-fed antibiotic growth promoters in Europe and other countries and the consumers concern for quality and safety of animal products being consumed, phyto-additives such as moringa leaf meal at 5g/kg diet may be considered as an alternative to antibiotics in promoting growth, quality and safety of meat of broiler chickens.

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ASSESSMENT OF SOME MACROMINERAL CONCENTRATION OF A GRASS/ LEGUME SWARD IN RELATION TO LIVESTOCK REQUIREMENTS

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Abstract

The assessment of macromineral concentration of Panicum/Stylosanthes mixtures was carried out at the Cattle Production Venture, Federal University of Agriculture, Abeokuta, in Southwest Nigeria. The study aimed to determine the concentration of some macromineral elements in the grass/legume pasture grazed by the animals with the view of recommending an application of relevant fertilizers. The experiment was laid out as a split-plot design with two spatial distribution of biomass (dense and sparse) assigned to the main plot and three cut back heights (10, 15 and 20 cm above ground) allotted to the subplot. Main plots measured 15 x 5 m while sub-plots were 5 x 5 m. Samples of *Panicum maximum* (*Ntchisi*) and *Stylosanthes guianensis* were clipped and analyzed to determine the concentration of P, K, Ca, and Mg. Results showed that both forage types had sufficient levels of P, K, Ca and Mg to meet the requirements of ruminants. Calcium (9.10 g kg -1) and P (3.75 g kg -1) contents of *P. maximum* were ($p<0.05$) higher for the dense biomass cut back to 15 cm height. *Stylosanthes guianensis* in the dense biomass cut to 15 cm recorded higher value for Ca than other treatments. Magnesium concentration was higher for *P. maximum* in the dense biomass (6.68 g kg -1) cut to 10 cm and the same pasture type cut to 15 cm height above ground recorded ($P<0.05$) higher value (5.41 g kg -1) for *S. guianensis*. Cutting of forages with the aim of promoting the regrowth of young shoots appears to favour sufficient accumulation of minerals in both forage types. Since only a portion of the total forage available in a sward is useful to grazing animals based on sward characteristics (e.g spatial distribution), herbage with higher quality is preferred by ruminants. It can be concluded that dense pastures are highly nutritive and cut back to 10 cm above ground could potentially produce regrowth herbage with sufficient macrominerals which relegate the need for mineral supplementation in grazing animals and fertilizer application to the pasture sward.

Keywords: Spatial distribution, *Panicum maximum*, *Stylosanthes guianensis*, cut back, grazing

ÉVALUATION DE CERTAINES TENEURS EN MACROMINERAUX D'UN TAPIS D'HERBES / GRAMINEES EN FONCTION DES BESOINS DES ANIMAUX

Resume

L'évaluation de la teneur en macrominéraux des mélanges de Panicum / Stylosanthes a été réalisée à la Cattle Production Venture de l'Université Fédérale d'Agriculture d'Abeokuta, dans le sud-ouest du Nigeria. L'étude avait pour objectif de déterminer la concentration de certains éléments macrominéraux dans les pâturages d'herbes / graminées fréquentés par les animaux en vue de recommander l'application d'engrais appropriés. L'expérience a été présentée selon un dispositif à parcelles divisées avec deux distributions spatiales de biomasse (dense et éparse) assignées à la parcelle principale et trois aires à hauteurs d'herbes réduites (10, 15 et 20 cm au-dessus du sol) attribuées à la sous-parcelle. Les parcelles

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principales mesuraient 15 x 5 m tandis que les sous-parcelles mesuraient 5 x 5 m. Des échantillons de Panicum maximum (Ntchisi) et de Stylosanthes hamata cv (Verano) ont été coupés et analysés pour déterminer la teneur en P, K, Ca et Mg. Les résultats ont montré que les deux types de fourrage avaient des teneurs assez suffisantes en P, K, Ca et Mg pour répondre aux besoins des ruminants. Les teneurs en Calcium (9,10 g kg -1) et en P (3,75 g kg -1) de P. maximum étaient plus élevées (p <0,05) pour la biomasse dense ramenée à 15 cm de hauteur. Stylosanthes hamata dans la biomasse dense coupée à 15 cm a enregistré une valeur plus élevée pour le Ca par rapport aux autres traitements. La concentration de magnésium était plus élevée pour P. maximum dans la biomasse dense (6,68 g kg -1) coupée à 10 cm et le même type de pâturage coupé à 15 cm de hauteur au-dessus du sol a enregistré (P <0,05) une valeur plus élevée (5,41 g kg -1) pour S. hamata. Le fait de couper les fourrages dans le but de favoriser les repousses semble favoriser une accumulation suffisante de minéraux dans les deux types de fourrage. Puisque seule une partie du fourrage total disponible dans une couche végétale est utile aux animaux de pâturage en fonction des caractéristiques de la végétation (par exemple, la distribution spatiale), les ruminants préfèrent les herbages de meilleure qualité. On peut en conclure que les pâturages denses sont très nutritifs et qu'une coupure de 10 cm au-dessus du sol pourrait produire des repousses ayant suffisamment de macrominéraux, ce qui relègue le besoin de supplémentation minérale chez les animaux de pâturage et d'application d'engrais aux pâturages herbeux.

Mots-clés : répartition spatiale, Panicum maximum, Stylosanthes hamata, réduction de hauteur, pâturage

Introduction

Ruminant animals are usually dependent on the feed they consume for their dietary nutrients. However, significant quantities of these elements could be obtained from water and soil (Khan *et al.,* 2009). McDowell and Arthington (2005) divided the elemental mineral sources of feeds into various feed stuff to include pasture plants, concentrates, and mineral supplements. Dost *et al.,* (1990) and Khan *et al.,* (2009) therefore concluded that the cost of mineral supplementation can only be minimized through detailed knowledge of supply and availability of minerals in forages and other feeds consumed by animals.

The mineral content of forages varies widely with soils owing to the implication of tropical soil for low nutrient status. Adequate consumption of forages and minerals from the various grazing resources available to ruminants would help to determine the level of mineral consumption, and perhaps, the need for mineral supplementation. However, stage of growth, cutting height, soil type and plant species are some of the factors that come into play when evaluating the mineral composition of pasture plants (McDowell *et al.,* 1983; Khan *et al.,* 2005). Inadequate supply of macromineral nutrients in plant base feedstuffs hampers the productivity of ruminants (Fardous *et al.,* 2011). For instance, calcium is a major component of the skeleton, which is also involved in blood clotting, muscle contraction and activation of enzymes. The deficiency of this element leads to weak and soft bones in young ruminants, reduced bone growth and performance of calves (David and Casssey, 2017). It is noteworthy that information on the total concentration of minerals in forages is essential, however, the bioavailability of these elements is also important which varies among animal and forage species. The combination of these factors makes it extremely difficult for livestock producers to determine the actual mineral status of their herd, their need and degree of supplementation required to achieve the optimal production (Dost *et al.,* 1990; Dost, 2001; Khan *et al.,* 2009). Hence, the need to be aware of the mineral concentration in forages grazed by ruminants.

In Nigeria as well as other tropical countries, the outcome of poorly managed livestock is malnutrition which becomes apparent through emaciation. Grazing animals in Nigeria majorly depends on poor quality forage species found in natural pastures, particularly during the offseason. Where supplementation is deemed fit, agro-industrial by-products and crop residues are used to provide supplemental energy and protein to the animals, neglecting the mineral imbalance

of such diets. Albeit the importance of energy and protein of a feed to animals, optimum production is only attainable with the adequate supply of minerals (McDowell, 1985; Khan et al. 2004).

The use of sown pastures for livestock production in Nigeria is still gathering momentum. Ruminants are rarely supplemented with minerals except occasionally and where production is on a large scale. Therefore, pastures remain the major source of minerals and forages seldom meets the elemental needs of livestock (Miles and McDowell, 1983). Most pastures in Nigeria lack proper management in terms of plant population and cutting height to promote qualitative herbage regrowth (Jimoh, 2014). Identification of biomass distribution and cut back heights that optimize the net forage accumulation and delay senescence and stem accumulation would favour the efficient accumulation of large quantities of highly nutritious forage (Pinto *et al.,* 2001, Carnevalli *et al.,* 2006) Thus, it is important to evaluate the macro-mineral concentration of forage plants used for livestock production (McDowell, 1992; Khan *et al.,* 2006), since these class of elements is needed in sufficient quantity for optimal productivity of livestock. This study aimed to determine the concentration of some macro-mineral elements in the grass/legume pasture grazed by animals with the view of recommending an application of relevant fertilizers, the need for supplementation and the level at which such intervention, as part of management strategy should be provided for an optimal productivity of ruminants.

Materials and Methods

Experimental Site

The experiment was carried out at the Cattle Production Venture (CPV) grazing land, Federal University of Agriculture, Abeokuta, Ogun State at latitude 7° 10' N, longitude 3° 2' E, and altitude 76 m above sea level in the derived Savannahh zone of South-Western Nigeria. The climate is humid with a mean annual rainfall of 1037 mm and temperature of about 34.7°C. Relative humidity ranges between 63 - 96% in the rainy season (late March-October) and 55 - 82% in the dry season (November-early March) with an annual average of 82% (Google Earth, 2015).

Pasture management

The pasture sward used for the experiment was established four years ago. The entire pasture size is 5 ha and is composed mainly of Panicum maximum (Ntchisi) and *Stylosanthes guianensis* which occur in irregular patterns. Although, other volunteer species such as *Centrosema molle* and *Calopogonium mucunoides* were also present in the pasture. Majorly, the pasture is used for supplemental grazing of selected high producing animals from the herd. The entire pastureland was observed for varying spatial distribution of pasture biomass which was hypothesized to be capable of influencing the macro-mineral concentration of the forages therein. The experiment was carried out by demarcating a section of the pasture to have dense and sparsely populated pastures. Each of the dense and sparse pasture areas measures 15 x 5 m^2 and constituted a block. Each block was further divided into three cells each of dimension 5 x 5 m^2. Thereafter, forage in each pasture type was cut back at 10, 15 and 20 cm above the ground, and left for four weeks before collection of data in November 2015. The second data collection exercise was carried out in December 2015. The height of cut back was ascertained using sward stick.

Treatment combination

The treatments comprised of dense pasture biomass cut to 10 cm, 15 cm, and 20 cm above ground and sparse biomass pasture also cut to 10 cm, 15 cm, and 20 cm height. The cut back of the pasture was carried out in mid-October, 2015. The six treatments were replicated three times to give eighteen subplots.

Herbage sample collection

Forage samples were clipped from the demarcated plots in November and December 2015. The forage samples collected included *P. maximum var Ntchisi*, *Stylosanthes guianensis*, *Calopogonium mucunoides* and *Centrosema.*

molle. The samples were enveloped, oven dried at 65°C until constant weight was obtained and subsequently milled to pass 1mm sieve for laboratory analysis. The samples used for laboratory analysis were *P. maximum Ntchisi* and *Stylosanthes guianensis* being the cultivated and dominant species in the experimental plots.

Experimental Design

The experiment was laid out as a split-plot design with two pasture spatial distributions (dense and sparse) assigned to the main plot and the three cut back heights (10cm, 15cm and 20cm) assigned to the sub-plot.

Mineral content determination

Mineral contents of the samples were determined by wet ashing using digestion method as described by Fick et al. (1979). Ca, P, K and Mg were detected by atomic absorption spectrophotometry (AAS) (Anon, 1980).

Statistical Analysis

All data collected were analyzed using the General Linear Model (GLM) procedure and the treatment means separated using Tukey Honestly Significant Difference in R statistics (R Core Team, 2015).

Results

Table 1 shows the main effect of sward height and spatial distribution of biomass on the macro mineral concentration of *Panicum maximum* (Ntchisi). The result revealed that the effect of sward height was not significant ($p>0.05$) on the values of Mg, Ca and P. The concentration of K for grasses cut back to 10 cm height was highest ($P>0.05$) (13.3 g kg $^{-1}$) while the least value was observed for the grass cut to 15 cm height (10.5 g kg $^{-1}$). Spatial distribution of biomass did not ($P>0.05$) affect K, Ca and P contents of the grasses. In contrast, Mg concentration was influenced by the spatial distribution of biomass with the dense pasture biomass recording higher value than its sparse pasture counterpart. There were significant ($P<0.05$) differences in the values of K, Mg, Ca and P as affected by interactions of sward height and spatial distribution of biomass (Table 2). The values recorded for K ranged from 9.5 g kg $^{-1}$ for pasture with dense biomass cut to 15 cm height to 13.6 g kg $^{-1}$ for those with sparse biomass cut to 10 cm height above ground. Values of K for other treatments were similar. Magnesium concentration was highest for the pasture with dense pasture cut to 10 cm height while the value for sparse pasture biomass cut to 15 cm height was the lowest. The Mg content in the pastures with dense biomass cut to 10 cm, 15 cm, and 20 cm height, as well as

Table 1: Main effect of sward height and spatial distribution of pasture biomass on some macro mineral concentration of Panicum maximum.

	Potassium	Magnesium	Calcium	Phosphorus
	g kg^{-1}			
Sward height (cm)				
10	13.3^{a}	6.6	8.6	2.2
15	10.5^{b}	6.1	7.6	3.7
20	10.9^{b}	6.3	7.0	3.3
SEM	0.57	0.16	0.44	0.29
Spatial distribution				
Dense	10.9	6.6^{a}	8.0	3.1
Sparse	12.3	6.1^{b}	7.4	3.4
SEM	0.51	0.13	0.42	0.26

$^{a,b,c:}$ *Means in the same column with different superscript are significantly different ($P<0.05$) SEM: Standard error of mean.*

Table 2: Interaction effects of sward height and spatial distribution of biomass on some macro mineral concentration of Panicum maximum.

Spatial Distribution	Sward Height (cm)	Potassium	Magnesium	Calcium	Phosphorus
		g kg^{-1}			
Dense	10	12.9^{ab}	6.7^{a}	8.7^{ab}	2.1^{b}
	15	9.5^{b}	6.6^{a}	9.1^{a}	3.8^{a}
	20	10.4^{ab}	6.5^{a}	6.6^{ab}	3.4^{a}
Sparse	10	13.6^{a}	6.5^{a}	8.5^{ab}	3.6^{a}
	15	11.8^{ab}	5.5^{b}	6.1^{b}	3.7^{a}
	20	11.5^{ab}	6.1^{ab}	7.5^{ab}	3.1^{ab}
SEM		0.65	0.16	0.51	0.35

^{a,b,c} Means in the same column with different superscript are significantly different (p<0.05) SEM: Standard error of mean.

Table 3: Main effects of sward height and spatial distribution of pasture biomass on some macro mineral concentrations of *Stylosanthes guianensis*.

	Potassium	Magnesium	Calcium	Phosphorus
	g kg^{-1}			
Sward height (cm)				
10	12.4	4.7	14.6^{a}	3.1
15	11.1	4.8	10.7^{b}	2.9
20	11.2	4.0	14.1^{a}	2.9
SEM	0.32	0.35	0.34	0.30
Spatial distribution				
Dense	10.8	5.4^{a}	10.5^{b}	2.5^{b}
Sparse	11.6	4.1^{b}	13.4^{a}	3.4^{a}
SEM	0.25	0.24	0.43	0.23

^{a,b,c} Means in the same column with different superscript are significantly different (P<0.05) SEM: Standard error of mean.

Table 4: Interaction effect of sward height and spatial distribution of biomass on macro mineral concentration of *Stylosanthes guianensis*.

Spatial Distribution	Sward Height (cm)	Potassium	Magnesium	Calcium	Phosphorus
		g kg^{-1}			
Dense	10	9.7^{bc}	4.7^{ab}	10.7^{b}	2.8
	15	10.8^{abc}	5.4^{a}	10.5^{b}	2.7
	20	8.7^{c}	4.0^{b}	9.3^{b}	2.3
Sparse	10	12.4^{a}	4.7^{ab}	14.6^{a}	3.3
	15	11.6^{ab}	3.7^{b}	10.9^{b}	3.2
	20	11.2^{ab}	4.0^{b}	14.1^{a}	3.6
SEM		0.22	0.24	0.50	0.20

^{a,b,c} Means in the same column with different superscript are significantly different (p<0.05) SEM: Standard error of mean.

sparse pasture biomass cut to 10 cm height, were similar. Ca significantly ranged from 6.1 g kg $^{-1}$ for sparse pasture biomass cut to 15 cm height to 9.1 g kg $^{-1}$ for dense pasture cut to 15 cm height. Similar values of calcium were recorded for both pasture type cut to 10 cm and 20 cm heights. Phosphorus values ranged ($P<0.05$) from 2.1 g kg $^{-1}$ for plots with dense pasture cut to 10 cm height to 3.6 g kg $^{-1}$ for those with dense pasture cut to 15 cm height. The phosphorus content of *P. maximum* was similar for all the plots cut to 15 and 20cm heights respectively.

Sward height did not affect the concentrations of K, Mg, and P in the clipped samples of *S. guianensis* (Table 3). The values recorded for Ca significantly ranged from 10.7 g kg $^{-1}$ for the legume in the pasture cut to 15 cm height to 14.6 g kg $^{-1}$ for that cut to 20 cm. The effect of spatial distribution of biomass was significant on the Mg, P and Ca contents of the legume. Pastures with dense biomass recorded higher value for Mg than others while sparse biomass was observed to have higher Ca and P values respectively.

Table 4 shows the interaction effect of sward height and spatial distribution of biomass on some macro mineral concentration of *Stylosanthes guianensis*. There was an interaction effect on all the macro minerals investigated except for P. The values obtained for Mg significantly ($P<0.05$) ranged from 3.7 g kg $^{-1}$ for plots with sparse pasture cut to 15 cm height to 5.4 g kg $^{-1}$ for those with dense pasture cut to 15 cm height. Sparse pasture plot cut to 15 cm and 20 cm height had statistically identical values. Calcium concentration was higher for sparse pasture plots cut to 10 cm height while the lowest value was observed for plots with dense pasture cut to 20 cm height. Plots with sparse pasture cut to 10cm height recorded higher value for K, and the lowest was observed for plots with dense pasture cut to 20 cm height.

Discussion

Phosphorus content recorded for *P. maximum* fell within the recommended level of (1 -4.8 g kg -1) required of different classes of ruminant animals as suggested by McDowell (1997). However, the concentration of P as influenced by the interaction of cut back height and spatial distribution of biomass differed, with sparse plot cut back at 10 cm recording the highest. These values compare well with those reported by Dele (2012) for *P. maximum Ntchisi* and Mustapha et al. (2015) for grasses in a natural pasture. The high P content recorded for the sparse plot could be attributed to reduced competition for nutrients among the plants, which invariably might have to pave way for better P uptake from the soil in the said treatment. Relative to the fact that tropical soils have long been implicated for a low level of phosphorus, the general high P level recorded for *P. maximum* in this study could be partly attributed to sward height, but more importantly to the mixture of the grass and legume in the sward.

The K concentration recorded in this study differed as influenced by cut back height and the values were above 8 g kg -1 recommended for grazing animals (Underwood 1981). However, McDowell (1985) reported that high producing animals may require K level of 10 g kg -1 particularly under stress which the grass in this study could provide. Similar K values were reported by Khan et al. (2009) when the mineral concentration of forages for grazing ruminants were evaluated in Pakistan. The interaction of the factors under study affected K concentration across the treatments with the highest observed for *P. maximum* in the sparse plot cut back at 10 cm height. The high K content for *P. maximum* in this treatment guarantees sufficient acid-base balance, regulation of osmotic pressure and water balance, as well as muscle contractions for grazing animals. However, where potassium is excessive with an attendant deficiency of sodium, the balance of all other cations and anions may be altered (Farhad, 2012). More importantly, an excess of potassium could interfere with uptake and availability of sodium, calcium, and magnesium in animal diets.

The Mg concentration obtained for *P. maximum* in both sparse and dense plot revealed

differences and were above the tolerable level of 4 g kg -1 reported by NRC (1980). These values were high enough to meet the Mg requirement of beef cattle (Khan et al. 2007), as well as growing lambs, lactating ewes, and goats (Meschy, 2000). With respect to the interaction of cut back height and spatial distribution of biomass, sufficient concentration of Mg was recorded across the treatments which indicate that enzyme activation and energy metabolism will not be impaired when the grass is consumed by grazing animals. The observed difference in the Mg concentration of *P. maximum* across the treatments could partly be explained by the level of Mg in the soil (Khan et al. 2009), cut back height, and proportion of morphological fractions collected during sampling. The availability of Mg in diets of stock is influenced by other mineral elements, particularly K. Fardhad (2012) noted that high dietary levels of K and N will inhibit Mg absorption from the rumen. Calcium concentration and soluble carbohydrates may respectively alter dietary Mg requirements of livestock, in as much as raised dietary P levels seem to lower Ca and Mg requirements (Dua and Care, 1995; Judson and McFarlane, 1998).

There was no marked effect of cut back height and spatial distribution of biomass on the Ca concentration of the grass in this study. However, Ca content was affected by the interaction of the factors to which the treatments were subjected. The recorded values for Ca in this study were above the values of 3.3 g kg -1 reported by Dele (2012) for *P. maximum Ntchisi* and 4.04 g kg -1 reported by Mustapha *et al.,* (2015) for natural pasture forages. These values also exceed the recommended Ca concentration of 1.8 – 8.2 g kg -1 for ruminant animals (McDowell, 1997), and this implies that the grass has sufficient Ca concentration to meet this requirement. In addition, it also surpasses the suggested Ca requirement (1.2-2.6 g kg -1) for maintenance of growing and lactating sheep (Reuter and Robinson, 1997), suggesting that blood clotting, muscle contraction and regulation of the heart could be well enhanced through consumption of the grass.

In the region of investigation, it could be presumed that legumes contain more mineral concentrations than would be in grass species in the wet season. The result from this study showed that mean calcium concentrations were adequate and sufficient. However, the requirement of Ca in the grazed herbage of ruminants is a subject of considerable debate and it is dependent on the animal type, level of production, age and weight (Farhad, 2012). Reuter and Robinson (1997) suggested Ca requirement for maintenance of growing and lactating sheep to be 1200 - 2600 mg kg -1. Thus the Ca content of *P. maximum* evaluated in this study was considered adequate for the optimum performance of ruminants. These results clearly showed that problems of Ca deficiency would not be anticipated.

Interaction of cut back height and spatial distribution of biomass was inherent in the K content of *S. guianensis* in this study. The values recorded across the treatments were above 8 g kg -1 recommended for grazing animals (Tejada et al. 1985). Therefore, problems of K deficiency are unlikely to surface in animals grazing the experimental field. Similar K concentrations have also been reported (Farhad, 2012). Reproductive losses appear to be further heightened when ruminant animals consume forages with high potassium and low sodium. Although sodium concentration was not investigated in this study, it suffices to state that K content of the legume was appropriate since excessive potassium in forages could induce a deficiency of other minerals, as well as immunity suppression.

Magnesium concentration of *S. guianensis* across the treatments were above the recommended requirement of ruminants (Islam *et al.,* 2003). Treatments with dense biomass across the various heights had slightly higher levels of Mg than those of sparse pasture biomass across the heights. These forages would, therefore, meet the theoretical requirement of Mg for beef cattle and lactating cows (1.2-2.1 g kg -1 DM) (Farhad, 2012). Plants do not contain adequate levels of phosphorus and magnesium in the wet season. Khan (2003) reported that the uptake of these minerals is retarded due

to cool wet conditions. This may justify the level of Mg observed in the legume since the experiment was conducted at the onset of the dry season. However, Farhad (2012) reported that cows depend on a frequent supply of magnesium from the feed since mobilization of magnesium from the bone is not very efficient.

Calcium concentration of *S. guianensis* in this study exceeds the range of (1.2 – 8 g kg -1) required for optimal performance of ruminant animals (McDowell, 1992; 1997). Forage Ca concentrations of 2 - 6 g kg -1, with higher requirements for lactation, have been variously recommended for cattle and sheep (Khan *et al.,* 2006; McDowell *et al.,* 1993; Farhad 2012). The observed variations in Ca level between findings in this study and values reported in the literature could be partly, due to different forage species, sward composition, and variations in soil characteristics due to pasture distribution on the experimental field. Assuming the collected forage samples represents the actual grazing resource of ruminants in the region of investigation, it is pleasing to infer that there is no need for calcium supplementation of ruminants grazing therein. In addition, calcium requirements are also dictated by factors such as age, weight, type, and level of production (Khan *et al.,* 2009). Growing animals absorb calcium more efficiently than older animals, owing to their high demand for calcium to satisfy rapid growth rate. Thus, forage Ca concentration observed in this study was considered apt for the optimum performance of ruminants. Phosphorus concentration in the legume was well within the values 1 - 4.8 g kg^{-1} recommended for optimal performance of ruminants (McDonald, 1997) and this is perceived to be the result of the beneficiary association between the grass and the legume in the plots.

Conclusion

This study shows that the macro-minerals investigated (Ca, P, K, and Mg) were present in an adequate amount for grazing animals in the experimental area. Given that tropical soils are well known to lack sufficient phosphorus, the beneficiary association of the grass/legume mixture resulted in improved P content in the forages, and this merit advocacy for grass/legume mixtures for animal feeding. Therefore all the forages would generally meet the listed macro-nutrient requirement of animals. Since selectivity by animals usually leads to consumption of herbage materials of somewhat higher quality relative to total available biomass, it is satisfying to conclude that potential macro-mineral deficiency in animals grazing in this area is not expected.

Recommendation

Based on the results from this study, cut back of Panicum/stylo sward at 10 cm height above ground level is recommended to meet the macromineral requirements of grazing livestock. Establishment of grass/legume mixture is recommended owing to the beneficiary association between grass and legume, capable of improving P levels in forages grazed or harvested from such swards to meet ruminant requirements.

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GASTROINTESTINAL PARASITES OF CATTLE AND SHEEP SLAUGHTERED AT GOMBE ABATTOIR, GOMBE STATE, NORTH-EASTERN NIGERIA

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Abstract

The objective of the study was to determine the prevalence and the distribution of gastrointestinal parasites of bile and faeces of cattle and sheep slaughtered at the Gombe abattoir, using bile sedimentation, faecal sedimentation and faecal floatation techniques. Composite samples were collected from three hundred and eighty nine (389) cattle and 398 sheep. An overall prevalence of 80.72% and 55.28% was respectively found in cattle and sheep. Two hundred and sixty one 261(67.07), 51(13.11%) and 272(80.71%) cattle and 55(13.81%), 28(7.03%) and 182(45.72%) sheep were positive for one parasite or the other by the bile sedimentation, faecal sedimentation and faecal floatation techniques respectively. For both species, obtained results did not vary significantly ($P>0.05$) based on the sex, age and breed. However, there was a significant variations ($P<0.05$) based on sample type in cattle and month of sample collection and sample type in sheep. In cattle, the distribution of the recovered parasites showed the predominance of *Dicrocoelium hospes* 235(41.89%), followed *Fasciola* spp. 114(20.32%) and strongyle 75(13.37%). In addition, 58(10.34%), 1(0.18%) and 1(0.18%) cattle were respectively infected with coccidia, *Schistosoma bovis* and mite eggs. Mixed infections due to *Fasciola* spp./*Dicrocoelium hospes* was found among 58(10.34%) animals, while nine 9(1.60%) cattle were co-infected with strongyle and coccidia. In sheep, single infection due to *Fasciola* ssp., *Dicrocoelium hospes*, strongyle, coccidia, *Toxocara vitulorum* and *Moniezia expansa* were found among 11(3.44%), 52(16.25%), 86(26.88%), 133(41.56%), 1(0.31%) and 1(0.31%) animals respectively, while mixed infection due to *Fasciola* spp./*Dicrocoelium hospes* was found in 4(1.25%) and strongyle/coccidia in 32(10.00%) animals.

Conclusively, the results revealed high prevalence in both species. The abundance of strongyle, coccidia, *Fasciola* spp. and *Dicrocoelium hospes*, in addition to other identified parasites was noted from this study. Future investigations may require the differentiation of species of parasites such as Fasciola, in view of their economic and public health significance.

Key words: Gastrointestinal Parasite, Gombe, Abundance, Prevalence, Cattle, Sheep,

PARASITES GASTRO-INTESTINAUX DES BOVINS ABATTUS A L'ABATTOIR DE GOMBE DANS L'ÉTAT DE GOMBE DANS LE NORD-EST DU NIGERIA

Resume

L'objectif de l'étude était de déterminer la prévalence et la distribution des parasites gastro-intestinaux de la bile et des excréments de bovins et ovins abattus à l'abattoir de Gombe, en utilisant la sédimentation biliaire, la sédimentation fécale et les techniques de flottation fécale. Des échantillons composites ont été prélevés sur trois cent quatre-vingt-neuf (389) bovins et 398 ovins. Une prévalence globale de 80,72% et 55,28% a été observée respectivement chez les bovins et les ovins. Cent soixante et

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un 261 soit (67,07), 51 (13,11%) et 272 (80,71%) des bovins et 55 (13,81%), 28 (7,03%) et 182 (45,72%) des moutons ont donné des résultats positifs pour un parasite ou un autre, respectivement par la sédimentation biliaire, la sédimentation fécale et les techniques de flottation fécale. Pour les deux espèces, les résultats obtenus n'ont pas montré de variation significative (P> 0,05) basée sur le sexe, l'âge et la race. Cependant, on a remarqué des variations significatives (P <0,05) basées sur le type d'échantillon chez les bovins et le mois de collecte des échantillons et le type d'échantillon chez les ovins. Chez les bovins, la distribution des parasites récupérés a montré la prédominance de Dicrocoelium hospes 235 (41,89%), suivie de Fasciola spp. 114 (20,32%) et de strongles 75 (13,37%). En outre, 58 (10,34%), 1 (0,18%) et 1 (0,18%) bovins étaient respectivement infectés par coccidia, Schistosoma bovis et des œufs d'acariens. Des infections mixtes dues à Fasciola spp./Dicrocoelium hospes ont été observées chez 58 animaux (10,34%), tandis que 9 (1,60%) bovins étaient co-infectés par des strongles et des coccidies. Chez les ovins, une seule infection due à Fasciola spp., Dicrocoelium hospes, aux strongles, aux coccidies, à Toxocara vitulorum et à Moniezia expansa ont été trouvées respectivement chez 11 (3,44%), 52 (16,25%), 86 (26,88%), 133 (41,56%), 1 (0,31%) et 1 (0,31%) animaux, tandis que l'infection mixte due à Fasciola spp./Dicrocoelium hospes a été trouvée chez 4 animaux (1,25%) et les strongles / coccidies chez 32 (10,00%) animaux.

En conclusion, les résultats ont révélé une forte prévalence chez les deux espèces. L'abondance de strongles, de coccidies, de Fasciola spp. et Dicrocoelium hospes, en plus des autres parasites identifiés, a été notée dans cette étude. Les recherches futures pourraient nécessiter une différenciation d'espèces de parasites telles que Fasciola, compte tenu de leur importance pour l'économie et la santé publique.

Mots-clés : parasite gastro-intestinal, Gombe, abondance, prévalence, bovins, ovins,

Introduction

Gastrointestinal parasitism is known to be wide spread in the tropics and temperate regions, where it causes profound direct and indirect losses in various livestock production systems. There have been reports of occurrence of gastrointestinal parasites in food (Morgan *et al.,* 2006; Khan *et al.,* 2010) and draught (Sultan *et al.,* 2014) animals, with varied burdens linked to local agro-climatic and management conditions. Reports from Kenya among calves, sheep and goats (Maichomo *et al.,* 2004), India in goats (Sorathiya *et al.,* 2017), Cameroun in sheep and goats (Mbuh *et al.,* 2008), Costa Rica in cattle (Jimenez *et al.,* 2007) and Nigeria among cattle (Adedipe *et al.,* 2014) have indicated that the phenomenon is of great magnitude. Similarly, the predominant species of gastrointestinal parasites recovered in previous studies have not been consistent in all cases. This ranged from *Haemonchus* spp. (Owhoeli *et al.,* 2014), *Eimeria* spp. (Jimenez *et al.,* 2007), *Fasciola hepatica* (Choubisa and Jaroli, 2013), Trichostrongyles (Yohannes *et al.,* 2013), *Nematodirus* spp. (Tramboo *et al.,* 2015) and *Moniezia* spp. (Allwin *et al.,* 2016) depending on the geographical locations and the host species. The type of samples and the analytical techniques employed for the determination of gastrointestinal parasites affects the type and distribution of parasites.

The Guinea Savannahh zone where Gombe State is located is typically grassland interspersed with woodlands, thereby serving to provide enough grazing land and pasture for cattle rearing (Adang *et al.,* 2015). With about 80% of the population of Gombe State engaged in farming, enough fodders and animal feeds are available for the majority Fulani herders who are engaged in cattle rearing (Diary 2012, Gombe State Government, Federal Republic of Nigeria). Despite these potentials for greater animal production, no enough studies were done on gastrointestinal parasitism of food animals in Gombe State. To our knowledge, apart from the recent report on the occurrence of helminths in donkeys sampled from Gombe State (Jajere *et al.,* 2014), no study has been conducted on gastrointestinal parasites of cattle and sheep, thereby leaving a wide gap in knowledge. Therefore, the present study examined prevalence and intensity of gastrointestinal parasites, as well as abundance of the various gastrointestinal genera/species available.

Figure 1: Map of Nigeria showing Sampling Location (Abattoir) as modified from the Administrative Map of Nigeria

Materials and Methods

Study Area

The Gombe central abattoir is located in Gombe town, and Gombe town is the capital of Gombe State, north eastern Nigeria. The town lies between latitude 10°081N and 11°241E and longitude 11°021N and 11°181E, while the State lies between Latitude 9°301 and 12°301N and Longitude 8°451 and11°451E should be replaced with The town lies between latitude 10°081N and 11°241E and longitude 11°021N and 11°181E, while the State lies between Latitude 9°301 and 12°301N and Longitude 8°451and 11°451E. (Gombe State Diary, 2012). The State covers a land area of 20,265 square km and has warm climate with temperatures not exceeding 30°C from March to May. The vegetation is that of Guinea Savannahh grassland with interspersed woodlands, which provides enough grazing land and pasture for cattle rearing.

Study Design

Cattle and sheep of different breeds slaughtered at the Gombe central abattoir were examined in a cross-sectional study. Samples were collected during dry (February

and March) and rainy (June) seasons of 2016 using a convenient sampling method.

Sample collection, transportation and analysis

One thousand, five hundred and seventy two (1572) samples in all were collected in the study consisting of 786 biles and 786 faeces. Species-wise, 389 each of faeces and biles were from cattle, while 398 faeces and 398 biles were from sheep. The samples were collected and analysed as described by Hansen and Perry (1994).

Data Analysis

Data generated were entered in Excel spread sheet and SPSS version 17 (Inc. Chicago, USA) used to calculate percentages and summarized into tables and charts. Chi square was used to test for association between sampled animals and the different variables. Values of ($p \leq 0.05$ was defined as significant).

Results

Prevalence and distribution of gastrointestinal parasites of cattle from Gombe central abattoir

Three hundred and fourteen (314) cattle were infected with one parasite or the other among the 389 sampled, giving an overall prevalence of 80.72%. There was no statistical significant variation ($p>0.05$) in the obtained based sex, age, breed and months of sample collection. However, male animals and the samples collected in the month of March had higher chance (odds ratio >1.00) of acquiring infection than their corresponding pairs (Table 1). In addition, 261(67.09%), 51(13.11%) and 120(30.84%) animals were positive for one

Table 1: Prevalence of Gastrointestinal parasites of Cattle from Gombe Abattoir, Gombe State

Parameter	No. Sampled	No. +ve(%)	X^2	P value	OR	CI at 95%
Sex						
Male	74	64 (86.49)	1.953	0.1623	1.664	0.8097- 3.420
Female	315	250 (79.37)				
Total	389	314 (80.72)				
Age						
Young	40	32 (80)	0.0148	0.9030	0.9504	0.4188- 2.157
Adult	349	282 (80.80)				
Total	389	314 (80.72)				
Breed						
RB	50	40 (80)	0.4944	0.7810	Ref	
Cross	2	2 (100)				
WF	337	272 (80.71)	0.01413	0.9054	0.9559	0.4542- 2.012
Total	389	314 (80.72)				
Month						
February	54	45(83.35)	1.503	0.4717	Ref	
March	67	57(85.07)			1.140	0.4270- 3.043
June	268	212(79.10)			0.7571	0.3491-1.642
Total	389	314(80.72)				
Technique						
Bile sedimentation	389	261(67.09)[a]		0.045		
Faecal sedimentation	389	51(13.11)[b]				
Faecal floatation	389	120(30.84)[c]				

Different superscripts in columns differed significantly (P<0.05)

Table 2: Distribution of Genera/species of gastrointestinal parasites of cattle slaughtered at Gombe abattoir, North-Eastern Nigeria

Genera/Species of Parasites	Prevalence: Number (%)
Fasciola spp.	114 (20.32)
Dicrocoelium hospes	235 (41.89)
Strongyle	75 (13.37)
Fasciola spp./*Dicrocoelium hospes*	68 (12.12)
Strongyle/coccidia	09 (1.60)
Coccidia	58 (10.34)
Schistosoma bovis	01(0.18)
Mite egg	01(0.18)
Total	561(100)
Distribution of Parasites Genera/Species based on the technique employed	
Bile sedimentation	
Fasciola spp.	89(22.87)
Dicrocoelium hospes	235(60.41)
Faecal Sedimentation	
Fasciola spp.	43(11.05)
Dicrocoelium hospes	02(0.51)
Coccidia	01(0.25)
Schistosoma bovis	01(0.25)
Strongyle	04(1.02)
Faecal Sedimentation	
Strongyle	71(42.66)
Coccidia	57(0.25)
Mite egg	01(3.33)

Table 3: Prevalence of Gastrointestinal parasites of Sheep from Gombe Abattoir, Gombe State, North-Eastern Nigeria

Parameter	No. Sampled	No +ve(%)	X^2	P value	OR	CI at 95%
Sex						
Male	74	37 (50.00)	1.024	0.3116	0.7705	0.4645 - 1.278
Female	324	183 (56.48)				
Total	398	220 (55.28)				
Age						
Young	26	15 (57.69)	0.06568	0.7977	1.111	0.4969- 2.484
Adult	372	205 (55.11)				
Total	398	220 (55.28)				
Breed						
Yankasa	386	211(54.66)	2.554	0.2789	Ref	
WAD	02	02(100.00)				

Parameter	No. Sampled	No +ve(%)	X^2	P value	OR	CI at 95%
Balami	10	07(70.00)			0.5167	0.1316- 2.029
Total	398	220(55.28)				
Month						
February	81	32(39.50)	12.41	0.0020	Ref	
March	161	102(63.35)			2.647	1.529- 4.584
June	156	85(54.48)			1.833	1.062- 3.164
Total	398	220(55.28)				
Technique						
Bile sedimentation	398	55(13.81)[a]		0.043		
Faecal sedimentation	398	28(7.03)[b]				
Faecal floatation	398	182(45.72)[c]				

Different superscripts in columns differed significantly (P<0.05)

Table 4: Distribution of Genera/species of gastrointestinal parasites from sheep slaughtered at the Gombe abattoir, North-Eastern Nigeria

Genera/Species of Parasites	Prevalence: Number (%)
Fasciola spp.	11(3.44)
Dicrocoelium hospes	52 (16.25)
Strongyle	86 (26.88)
Coccidia	133 (41.56)
Toxocara vitulorum	01(0.31)
Moniezia expansa	01(0.31)
Fasciola spp./Dicrocoelium hospes	04 (1.25)
Strongyle/coccidia	32 (10)
Total	320 (100)
Distribution of Parasite genera/species based on Techniques used	
Bile sedimentation	
Fasciola spp.	11(2.76)
Dicrocoelium hospes	12(12.06)
Faecal sedimentation	
Dicrocoelium hospes	05(2.56)
Strongyle	07(1.25)
Coccidia	02(0.50)
Toxocara vitulorum	01(0.25)
Faecal floatation	
Coccidia	132(33.16)
Strongyle	81(20.25)
Moniezia expansa	01(0.25)

parasite or the other by bile sedimentation, faecal sedimentation and faecal floatation techniques respectively.

The distribution of recovered parasites in cattle are presented in Table 2. Single infections due to *Fasciola* spp., *Dicrocoelium dendriticum*, strongyle, coccidia, *Schistosoma bovis* and mite eggs were observed in 114(20.32%), 235(41.89%), 75(13.37%), 58(10.34%), 01(0.18%) and 01(0.18%) animals respectively. Among the 389 cattle examined, the recovery rate based on the techniques employed showed that, 89(22.87%) and 235(60.41%) animals were respectively infected with *Fasciola* spp. and *Dicrocoelium dendriticum* as determined by bile sedimentation method (Table 2).

Prevalence and distribution of gastrointestinal parasites of sheep from Gombe central abattoir

Table 3 present the results of the prevalence of gastrointestinal parasites of sheep in Gombe central abattoir. An overall prevalence of 220(55.28%) was obtained among 398 sheep examined in the study. No statistical significant variations ($p>0.05$) were observed in the obtained results based on the sexes, ages and breeds of animals. However, younger sheep and the samples collected in March and June had increased odds of acquiring infection than their corresponding counterparts. The following prevalence; 55(13.81%), 28(7.03%) and 182(45.72%) were obtained by bile sedimentation, faecal sedimentation and faecal floatation techniques. Coccidia led the table of mono-infected animals with 133(41.56%) prevalence (Table 4).

Discussion

Gastrointestinal parasitism remains an important impediment to optimal production in livestock production systems in the tropics, ostensibly due to lack of access to veterinary care and the management practices adopted. In this study, we report an overall prevalence of 80.72% and 55.28% respectively in cattle and sheep, higher than the previous observations of Khan *et al.,* (2010) among sheep, goats, buffaloes and cattle in Pakistan and Sorathiya *et al.,* (2017) among traditionally maintained goat flocks from South Gujarat. Higher results than the present finding have equally been reported (Mbuh *et al.,* 2008; Martinez-Valladares *et al.,* 2013; Squire *et al.,* 2013). However, our finding is similar to previous results obtained by Maichomo *et al.,* (2004) in sheep and goats in Kenya and Jyoti *et al.,* (2014) in buffaloes from Punjab. This similarity may probably be due to the effect of agro-climatic factors, as both study locations are situate in tropical climatic zones, which ensures the abundance of intermediate hosts and infective forms of parasites in the environment. In fact, prevalence of gastrointestinal parasites like *Fasciola* spp. can be predicted, where the population dynamics of the snail intermediate hosts is predictable (Radostits *et al.,* 2006). Jyoti *et al.,* (2014) reported that, diverse agro-climatic conditions, animal husbandry practices and pasture management have shown to largely affect the incidence and severity of various parasitic diseases in a region. The observation of significantly ($p>0.002$) infected sheep in the months of March and June than in February, agreed with the observation made previously (Tramboo *et al.,* 2015), where higher prevalence was recorded in summer and spring than in winter and autumn. This may probably be due to the period of favourable environmental conditions for the development of the infective forms and the intermediate hosts of parasites.

The predominant parasites genera/species recovered from cattle consisted of *Dicrocoelium hospes* and *Fasciola* spp., which belong to the class trematoda, while from sheep, coccidia and strongyle predominated the recovery list. This recovery may not be unconnected with the types of technique employed and samples collected, in addition to local climatic factors. Choubisa and Jaroli (2013) and Squire *et al.,* (2013) reported higher prevalence of *Fasciola hepatica* among other recovered parasites from cattle, buffaloes, sheep and goats in India and the preponderance of *Fasciola* spp. and *Dicrocoelium dendriticum* among cattle from Southern Ghana. Similarly, the recovery of coccidia and strongyle as leading parasites species from sheep is in agreement with the previous results (Yadav *et al.,* 2006;

Jatau *et al.,* 2011; Ibukun and Oludunsin, 2015), from India and North-Central Nigerian city of Minna. This may be related to the management system in practice, and under which most sheep are reared in the study locations. The practice of keeping sheep under semi-intensive system of management in the study area, where the faeces and urine mix together and become damp, can serve as favourable environment for the sporulation of coccidial oocysts. This may be responsible for the relative higher prevalence of coccidia in sheep than cattle.

The recovered parasites in cattle (*Fasciola* spp., *Dicrocoelim hospes*, strongyle, coccidia, *Schistosoma bovis* and mite egg) are similar to the previously reported among cattle and goats (Barua *et al.,* 2009) and in cattle from Bangladesh (Sardar *et al.,* 2006). However, more parasites Genera/species were recovered in the current study than the previous (Sardar *et al.,* 2006; Barua *et al.,* 2009), and may be justified by the fact that, both bile and faeces were sampled in this study, coupled with the employment of both floatation and sedimentation techniques for sample analysis. Coccidia, strongyle, *Dicrocoelium hospes* and *Fasciola* spp. were the common parasites recovered from sheep, similar to previous recoveries made by Biu *et al.,* (2009) and Kantzoura *et al.,* (2012). Although, parasites such as *Nematodirus* spp. recovered by Kantzoura *et al.,* (2012), were not found in this study, the finding of strongyle/coccidia mixed infections in 32(10.00%) animals attest to the importance of these parasites species in sheep within the study locations. Additionally, the presence of *Toxocara vitulorum* and Moniezia *expansa* in the present study, gives credence to the fact, these parasites are present and are worthy of note especially in future epidemiological work in the study location. Conclusively, although the species of *Fasciola* were not differentiated in this study, future epidemiological investigations in the study area may require differentiation of the species, in view of public health importance of mainly *Fasciola hepatica* and rarely *F. gigantica*.

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SINGLE *NUCLEOTIDE POLYMORPHISMS* IN TWO TLR GENES AND THEIR EFFECTS ON *TRYPANOSOMOSIS* TRAITS IN MUTURU AND WHITE FULANI CATTLE CHALLENGED WITH *TRYPANOSOMA VIVAX* IN NIGERIA

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Abstract

Trypanosomosis is a parasitic infection of man and animals caused by Trypanosoma spp. which occurs throughout the tropical regions especially in sub-Saharan Africa and the disease is a major constraint in cattle production. Toll-like receptors genes are crucial in triggering an innate immune response against pathogens. The study investigated single *nucleotide polymorphisms* (SNPs) in two toll-like receptors (TLR) 1 and 2 genes in Muturu and White Fulani cattle challenged with *Trypanosoma vivax* to determine their association with indices of trypanotolerance in these cattle breeds. Thirteen yearling Muturu and White Fulani bulls were used for this study. Four animals of each breed were intravenously inoculated with 2 x 106 trypanosome organisms per millilitre, while five animals served as uninfected control group. Data on haematological indices were collected twice weekly before and after *Trypanosoma vivax* inoculation for six weeks. A total of 16 SNPs were detected in the two TLR genes, out of which 7 were detected in TLR1 and nine in TLR2. Of the SNPs detected, 43.75% (n = 7) were transition and 56.25% (n = 9) were transversion. The *trypanosomosis* traits that were mostly associated with the TLR SNPs were WBC and PCV. The study concluded that 12TLR-SNPs in Muturu and White Fulani cattle were associated with WBC, 7 with PCV, 3 with RBC, 2 with eosinophil and none with monocyte. The significant relationships between TLR-SNPs and *trypanosomosis* traits if genotyped could provide important information that can be used in the control of the disease.

Keywords: Toll-like receptors, single *nucleotide polymorphisms*, *trypanosomosis*, cattle

POLYMORPHISMES MONONUCLEOTIDIQUES DANS DEUX GENES DE TLR ET LEURS EFFETS SUR LES TRAITS DE TRYPANOSOMOSE CHEZ DES BOVINS MUTURU ET FULANI BLANC EXPOSES AU TRYPANOSOMA VIVAX AU NIGERIA

Resume

La trypanosomose est une infection parasitaire de l'homme et des animaux causée par Trypanosoma spp., qui est présente dans toutes les régions tropicales, en particulier en Afrique subsaharienne, et la maladie est une contrainte majeure à la production bovine. Les gènes des récepteurs de type Toll (récepteurs TLR) sont cruciaux pour déclencher une réponse immunitaire innée contre les pathogènes. L'étude a examiné les polymorphismes mononucléotidiques (SNP) de deux gènes des récepteurs TLR 1 et 2 chez des bovins Muturu et Fulani blancs infectés avec Trypanosoma vivax en vue de déterminer leur association avec les indices de trypanotolérance chez ces races de bovins. Treize jeunes Muturu et Fulani blanc âgés d'un an ont été utilisés pour cette étude. Quatre animaux de chaque race ont été inoculés par voie intraveineuse avec 2 x 106 organismes trypanosomes par millilitre, tandis que cinq animaux ont servi de groupe témoin non infecté. Les données sur les indices hématologiques ont été recueillies deux fois par semaine avant et

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après l'inoculation avec Trypanosoma vivax pendant six semaines. Un total de 16 SNP ont été détectés dans les deux gènes TLR, dont 7 détectés dans TLR1 et neuf dans TLR2. Parmi les SNP détectés, 43,75% (n = 7) étaient des transitions et 56,25% (n = 9) des transversions. Les traits de trypanosomose principalement associés aux SNP TLR étaient le WBC et le PCV. L'étude a conclu que 12 TLR-SNP chez les bovins Muturu et Fulani blanc étaient associés aux WBC, 7 au PCV, 3 au RBC, 2 aux éosinophiles et aucun aux monocytes. Les relations significatives entre les TLR-SNP et les traits de trypanosomose - s'ils sont génotypés - pourraient fournir des informations importantes susceptibles d'être utilisées dans le contrôle de la maladie.

Mots-clés : récepteurs TLR, polymorphismes mononu-cléotidiques, trypanosomose, bovins

Introduction

Single *nucleotide polymorphisms* refer to a single base substitution in the genome sequence between members of species or between chromosomes in an individual at a given location. SNPs are abundantly distributed genetic variations in the genome and serve as valuable tools of interest in genetic selection due to their association with quantitative trait loci (QTL). Also the low mutation rate in SNPs from generation to generation makes it superior markers for studying complex genetic traits (Adebambo, 2010). High usage of next generation sequencing technologies for genotyping might substitute other molecular markers in the nearest future with SNPs (Semagn *et al.,* 2014). Several studies have shown that SNPs within TLR genes in cattle and humans seem to be associated with diseases (Pandey *et al.,* 2006).Mammalian TLRs play relevance role in innate and adaptive immune responses by detecting microbial molecular components which enable the host to recognize pathogen associated molecular patterns derived from wide range of microbes such as bacteria, viruses, parasites and fungi (Akira *et al.,* 2006). The innate immune system is the host's first line of defense against infection; therefore, its main role is to recognize invading pathogens early and trigger an appropriate proinflammatory response (Medzhitov and Janeway, 2000). The cells of the immune system recognize pathogens through TLR and TLR stimulation results in production of cytokines including interleukins, chemokines and interferons that steer the host's immune system into a cytotoxic, humoral, cell-mediated, or allergic response (Commins *et al.,* 2010). The sequence variations in TLR can influence how an organism develops and response to the environmental factors such as toxic or threat of disease (Akira *et al.,* 2006). The Nigeria livestock industry is a major contributor to the gross domestic products but diseases have been a hindrance to livestock productivity and one of such diseases is *trypanosomosis*. *Trypanosomosis* is a neglected tropical disease caused by protozoan parasite in the family Trypanosomatidae. The disease is a major impediment to agriculture affecting cattle, humans and other wild range of host in sub Saharan (Kahn, 2005; Noyes *et al.,* 2011; Talabi *et al.,* 2012; Biyazen, 2014). The most virulent and pathogenic trypanosome species responsible for this disease in cattle across the continent are *Trypanosoma vivax*, Trypanosoma congolense, Trypanosoma brucei. *Trypanosomosis* is transmitted cyclically by several species of tsetse flies (Glossinaspecies) and mechanically by other biting flies (Duffy, 2012; Takeet *et al.,* 2013). The virulence of the disease differs among host with antigenic variation being a limiting criterium to vaccine development Also, difficulty in the eradication of tsetse flies and problems associated with drug resistance have been great challenges in the control of *trypanosomosis* (Sekoni *et al.,* 2004; Gillingwater *et al.,* 2010). Hence, a promising approach and cost effective control that can tremendously reduce the impact of the disease on cattle productivity is genetic selection and development of trypanotolerant breeds of cattle that are able to response to environmental changes. Muturu an endangered cattle breed has the trypanotolerant genetic resource that can be conserved using molecular marker.

Materials and Methods

A total of thirteen yearling bulls comprising six Muturu and seven White Fulani cattle were used for this study at Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The animals were treated routinely against endo and ecto parasites and were allowed 8 hours of grazing daily (9.00am-5.00pm). The feeding was supplemented with dried cassava peels, salt lick and clean water (ad libitum) was provided. They were stabilized for two months thereafter blood and faecal samples were screened to obtained baseline information prior to *Trypanosoma vivax* challenge. *Trypanosoma vivax* used for this study was obtained from the blood samples of natural infected cattle slaughtered at Lafenwa Abattoir, Abeokuta, Ogun State. The blood samples were collected from the jugular vein into vacutainer tubes containing Ethylene Diamine Tetracetic Acid (EDTA) and transported in a portable refrigerator to the Veterinary Parasitology Laboratory of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, for parasitic analyses. The *Trypanosoma vivax* was identified based on movement, morphology and PCR technique. About 0.5ml of blood with *Trypanosoma vivax* was passaged intravenously into an apparently healthy Red Sokoto goat which served as donor. The goat was monitored for a week to ensure preservation and development of parasites after which, 1ml of the blood containing 2 x 106 trypanosome organisms per milliliter from the donor goat was inoculated into each cattle via the jugular vein. Four animals of each breed were challenged with *Trypanosoma vivax* through the jugular vein while the other animals served as the uninfected group (negative control).Blood samples for haematological parameters were taken from these animals before challenged and at three days interval after the challenge for the duration of six weeks. Routine haematological parameters consisting of the Packed Cell Volume (PCV) was determined using micro-haematocrit method as described by Talabi (2006), erythrocyte count (red blood cell), leukocyte count (white blood cell) were determined after blood collection using improved Neubauerhaemocytometer as described by Jain (1986). Differential leukocyte counts were determined by scanning Giemsa stained slides as described by Schalm et al. (1975). DNA was isolated from the blood using the ZymoBeadTM Genomic DNA kit (Zymo Research Corporation, Irvine, California, U.S.A.) following the manufacturer's protocol. The forward and the reverse primer sequences for each of the chosen primers were labelled from 51 to 31. These primer sequences were obtained from the work of Tirumurugaan et al. (2009) and Wheto (2012) as presented in Table 1. PCR was performed using 4µl genomic DNA in a 20µl reaction volume in master mix containing 0.4MTris–HCl, 0.1M (NH4)2 SO4, 0.1% w/v Tween-20, 7.5mM MgCl2, 1 x PCR buffer comprising 1.5mM MgCL2, 1mM dNTPs and 1.25µ Taq DNA polymerase (Solis, Biodyne, Estonia, Europe). PCR was performed using the bovine-specific TLR1 and 2 primers as listed in Table 1 under the following conditions: Initial denaturation at 94oC for 4 minutes, 30 cycles of denaturing at 94oC for 1 minute, annealing (temperature specific for each TLR gene) for 1 minute, initial extension times at 72oC for 1 minute, followed by final elongation at 72oC for 10 minutes in a thermocycler (Biorad, Hercules, CA, U.S.A.). Five microlitres of the PCR products were electrophoresed through 1% agarose gel containing 0.5µg/ml gel red.After electrophoresis, PCR products were viewed under ultraviolet light using a transilluminator and scored using GeneMate Quanti-Marker 100bp DNA ladder as Standard Marker (BioExpress, Kaysville, UT, USA). The presence of the fragments of interest was verified and then a photograph was taken showing samples and marker in their lanes.

Sequencing of the amplified fragments was carried out with Applied Biosystems Automated 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) at Cornell University, Ithaca, USA. Bioedit software (version 5.0.9; Hall, 1999) was used to trim and edit each of the sequences. A consensus sequence for each TLR gene was determined using CodonCode Aligner software (http;//

www.codoncode.com/aligner). The consensus sequence was blasted against Nucleotide database in NCBI using BLASTn for percent identity with database TLR nucleotides. TLR single *nucleotide polymorphisms* (SNPs) were identified using CodonCode Aligner software. Each sequence in the genes was translated into amino acid sequence using NCBI ORF Finder software (www.ncbi.nlm.nih.gov/gorf.html) in order to study results of SNPs on the Amino acids.

Statistical Analyses

A preliminary analysis in which estimates of allele and genotypic frequencies of the SNPs were computed using genetic package in R (version 3.02) and then tested for deviation from Hardy-Weinberg Equilibrium according to Chi-square test and level of significance at 1 degree of freedom but the results were not included here. Effects of SNPs and breed on haematological parameters were analysed with "R" software (www.r) using Generalized Linear Model in lme4 package in R. Initial haematological parameters were used as covariate in the model. The model equation was given below:

$$Y_{ijk} = \mu + A_i + \beta + B_j + _{ijk}$$

Where:
Y_{ijk} = individual observed trait of interest
μ = population mean
β = covariate effect
A_i = effect of ith breed (i = Muturu and White Fulani)
B_j = effect of jth SNP genotype within TLR gene (j = 1, 2)
$_{ijk}$ = residual error

Interaction effect was not significant and so, was removed from the model.

Results

A total of sixteen SNPs were detected. Seven SNPs were detected in TLR 1 and nine SNPs were detected in TLR 2. Nine of the SNPs detected were transversion SNPs and seven were transition SNPs as presented in Table 2. The SNPs detected in TLR1 were in the non-coding region except one in the coding region with non-synonymous (A181G; Glutamine >Lysine) mutation while in TLR 2 one non-synonymous SNPs (C464G; Serine > Tryptophan) occurs in the coding region. Analyses of the effects of SNPs on haematological parameters were done to determine the relationship between the five traits and the nucleotide changes in the TLR1 and TLR2 genes. Six of the TLR1 SNPs, and six of the TLR2 SNPs were observed to be significantly associated with WBC (n=12). One out of the TLR1 SNPs, and six of the TLR2 SNPs were also observed to be associated with PCV (n=7), three of the SNPs were significant association with RBC; two SNPs in TLR1 one in TLR2 SNPs, two SNPs in TLR1 were significantly associated with eosinophil and none was significantly associated with eosinophil.

Discussion

DNA sequencing of TLR genes of Muturu and White Fulani cattle revealed a total of sixteen (16) SNPs with 43.75% (n=7) transition (A/G, C/T) and 56.25% (n=9) transversion (A/C, A/T, G/C and G/T). The nucleotide differences detected in TLR1and TLR2 indicate more variations at these loci which could be used as markers in selection for disease resistance in livestock. Konnai *et al.,* (2003) reported that polymorphisms and mutations ingenes may result to variation in disease resistance and can also be used to detect traits that may aid selection for cattle. TLR1 with lower polymorphic sites suggest low level of variation at this locus in the cattle used (Marco *et al.,* 2009 and Ilori, 2015). The total number of SNPs (16) detected in this study for TLR1 and TLR2 was higher than that reported by Wheto (2012) who observed a total of 7 SNPs for TLR1 and TLR2 genes in Nigerian goats. These differences may reflect high pathogen diversity and might also be attributable to the species of animals studied. Dubey *et al.,* (2012) reported variations in Leucine Rich Repeats (LRRs) in different TLRs of various species and that the number and structure of LRRs present

Table 1: Toll-Like Receptor Primer Sequences for SNPs Identification

TLR	Primer sequence 51-31 forward/reverse	PCR amplicon size (bp) *	Annealing temperature* (°C)
TLR1	CTGCCCATATGCCAAGAGTT AAACCAACTGGAGGATCGTG	389	55.6
TLR2	GCTCCTGTGACTTCCTGTCC CCGAAAGCACAAAGATGGT	467	55.3

*Source: Tirumurugaan et al,. (2009), Wheto, (2012), bp: basepair, *Field Results*

Table 2: Single *Nucleotide Polymorphisms* detected in Two TLR genes in Muturu and White Fulani Cattle

Gene	CHR Number	SNP Position	SNP Category	Start Position	Stop Position	Number of SNPs
TLR1	6	Muturu		83	383	4
		T11A	Transversion*			
		C48A	Transversion*			
		A49G	Transition*			
		T51G	Transversion*			
		White Fulani				3
		T19A	Transversion			
		G22A	Transition			
		A181G	Transition**			
TLR2	17	WF		13	464	9
		C58A	Transversion			
		G72A	Transition			
		C73T	Transition			
		A75C	Transversion			
		G76A	Transition			
		G78C	Transversion			
		C123A	Transversion			
		G264A	Transition			
		C464G	Transversion**			

**Synonymous, ** Non-synonymous*

in the ecto-domain of TLRs significantly alter the function of pathogen recognition receptor system within a species. There was no SNP detected in TLR2 gene in Muturu cattle indicating that the locus may be an important region for information about resistance to *trypanosomosis*. Takeda and Akira (2005) reported that TLR2 recognises a variety of molecules such as glycosylphosphatidylinositol from trypanosome species, zymosan from yeast, peptidoglycan and lipoiteichoic acid derived from Gram-positive bacteria. A polymorphism in TLR has been associated with increased susceptibility to infection with Staphylococcus aureus (Lorenz *et al.,* 2000; Ogus *et al.,* 2004). All the polymorphisms in the coding region of the TLR genes studied are SNPs of which two are non-synonymous and others are synonymous amino acids. The remaining SNPs occurred in the non-coding region of the TLR. The non-synonymous coding SNPs (nsSNPs) might be responsible for protein single point mutation which may be neutral or disease associated (Capriotti *et al.,* 2006) and probably

Table 3: Effects of TLR SNPs on haematological traits

SNP Position	Monocyte (%)	Eosinophil (%)	WBC (×109/L)	RBC (×1012/L)	PCV (%)
T11A	4.11 ±0.31	1.13 ± 0.52	8.07 ±0.12[a]	7.60±0.06[a]	28.96±0.21
C48A	2.76±0.50	0.26 ± 0.21	6.32 ± 0.21[b]	6.83 ± 0.05[b]	28.83 ± 0.23
A49G	2.92 ±0.13	0.84±0.08[b]	7.04 ± 0.16[b]	7.01 ± 0.11	28.91 ± 0.41
T51G	3.056±0.19	0.89 ± 0.12[a]	7.36±0.23[a]	7.11 ± 0.17	29.00 ± 0.61
T19A	4.33 ± 0.31	1.19 ± 0.52[a]	8.09 ± 0.37[a]	7.68 ± 0.06	29.23 ± 0.21
G22A	2.76 ±0.50	0.26 ± 0.21[b]	6.32 ± 0.21[b]	6.83± 0.06	28.83 ± 0.23
A181G	2.97 ±0.14	0.93 ± 0.09	7.01 ± 0.20***	7.09±0.12***	28.85 ± 0.40*
C58A	3.45 ± 0.48	0.81 ± 0.22	8.50 ± 0.30	6.98 ± 0.28	29.26 ± 1.43
G72A	3.07 ± 0.36	0.61 ± 0.15	7.49 ± 0.40	6.89 ± 0.34	28.85 ± 1.22
C73T	2.83±0.13	0.95 ± 0.09	7.02±0.19b***	6.95 ± 0.11	28.50±0.41*
A75C	3.45 ±0.13	0.81 ± 0.09	8.50±0.19a***	6.98 ± 0.11	29.26 ± 1.41*
G76A	3.45±0.48	0.81 ± 0.22	8.50±0.30	6.98 ± 0.28	29.26±1.43*
G78C	3.45±0.48	0.81 ± 0.23	8.50±0.30	6.98 ± 0.28	29.26 ± 1.43*
C123A	3.45 ± 0.48	0.81 ± 0.22	8.50±0.30	6.98 ± 0.28	29.26 ± 1.43*
G264A	3.45 ±0.48	0.81 ± 0.23	8.50±0.30	6.98 ± 0.28	29.26±1.43*
C464G	2.94 ±0.13	0.94 ± 0.09	7.15 ± 0.18	6.92 ± 0.11*	28.56 ± 0.40
Total significant	0	2	12	3	7

**P<0.05, ***P<0.001*

result in an attenuated response to a variety of microbial agonists (Omueti *et al.,* 2007). The nucleotide substitutions that alter the amino acid in the TLR genes may affect ligand binding because TLRs have extracellular domain responsible for PAMP-binding and intracellular domain which binds signalling molecules and initiate immune responses (Muzio *et al.,* 2000; Cargill and Womack, 2007). The *trypanosomosis* traits that were mostly highly associated with the TLR SNPs were WBC, RBC, PCV and eosinophils. According to Yue (2014), SNPs serve as suitable markers for linkage mapping and marker assisted selection of important traits if they are closely-linked to the traits. TLR2 SNPs had the most frequent nucleotide changes associated with WBC and PCV while effects of TLR1and TLR2 SNPs on monocytes were not significant. This result is in line with previous study of Muchaet *et al.,* (2009) who detected association for which TLR variation decreased the risk of Mycobacterium avian subspecie paratuberculosis infection in bovine. Similarly, genetic variability in TLR2 and TLR4 has been associated with mastitis in cattle (Huang *et al.,* 2011 and Sharma *et al.,* 2006). In conclusion, Sixteen SNPs were detected in TLR1 and TLR2 genes in Muturu and White Fulani cattle. TLR1 and TLR2 SNP results may provide important information on the genetics of susceptibility/resistance to *trypanosomosis* and likely contain candidate SNPs responsible for the observed phenotype. SNPs in TLR1 and TLR2 were associated with *trypanosomosis* traits in Muturu and White Fulani cattle and these need validation to be used as markers in the selection of cattle with resistance to *trypanosomosis*.

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The Animal Care and Use Committee of the Federal University of Agriculture, Abeokuta, Nigeria, approved all the procedures used for the research.

Conflict of interest:

Authors declare no conflict of interest.

Compliance with ethical standards:

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CURRENT ATTITUDES REGARDING THE USE OF PERIOPERATIVE ANALGESICS AND ROUTINE ANAESTHETIC MANAGEMENT IN DOGS AND CATS BY VETERINARIANS IN CAMEROON

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Abstract

A survey was performed in 2017 to evaluate the use of perioperative analgesia and routine anaesthetic management in dogs and cats by Cameroon veterinarians in 19 veterinary clinics, including 7 and 12 in Douala and Yaounde, respectively. The questionnaire consisted of five sections recording demographic data, attitudes towards pain assessment and use of main analgesics in daily practices. Information about premedication, induction, maintenance and complications associated with *anaesthesia* in dogs and cats was also obtained. Only 15.79% of surveyed veterinary surgeons had undergone specialization in surgery and 36% considered their methods of pain quantification and control to be inadequate. The major part of the studied population (52.63%) was above 40 years old. Evaluated by the behavioural indicators (94.73%), animal pain is usually not quantified. The most used analgesics were *glucocorticoids* (*dexamethasone*, *prednisone*) and non-steroidal anti-inflammatory drugs (*phenylbutazone*, tolfenamic acid and *meloxicam*). The percentage of animals receiving analgesics postoperatively was 88.99% in dogs and 79.70% in cats. Few patients (28.72%) were weighed before applying *anaesthesia*. The most common monitored parameters were heart (78.95%) and respiratory (73.68%) rates. The use of *anaesthesia* and the choice of analgesic molecules varied significantly from one town to another ($p = 0.00$), and from dogs to cats ($p = 0.03$). Acepromazine was the most common used premedication in dogs and cats. Ketamine was the induction agent frequently used in dogs and cats. The complications that veterinarians encountered during *anaesthesia* were awakening and cardiorespiratory arrest. The anaesthetic death rate was 0.63%. Some improvements, particularly during pre-anaesthetic examinations and the use of perioperative drugs (analgesia and *anaesthesia*) can reduce the incidence of complications.

Keywords: Pain, *anaesthesia*, analgesics, survey, cats, dogs, monitoring, Cameroon.

ATTITUDE DESVÉTÉRINAIRESVIS-À-VIS DE L'UTILISATION PÉRIOPÉRATOIRE DES ANALGÉSIQUES ET SURVEILLANCE ANESTHÉSIQUE CHEZ LES CHIENS ET CHATS AU CAMEROUN

Résumé

Une étude a été menée en 2017 afin d'évaluer l'utilisation périopératoire des analgésiques et la pratique de l'anesthésique chez les chiens et les chats par des vétérinaires privés du Cameroun. La collecte des données s'est faite dans 19 cliniques vétérinaires, dont 7 à Douala et 12 à Yaoundé. Le questionnaire était composé de cinq sections récoltant les données démographiques, les attitudes vis-à-vis de l'évaluation de la douleur et l'utilisation des principaux analgésiques dans la pratique quotidienne. Des informations sur la prémédication, l'induction, l'entretien et les complications associées à l'anesthésie chez les chiens et les chats ont également été obtenues. Seuls 15,79% des vétérinaires interrogés avaient subi une spécialisation en chirurgie et 36% ont considéré leurs méthodes de quantification et de contrôle de la douleur comme étant inadéquates. La majeure partie de la population étudiée (52,63%) était âgée de plus de 40 ans. Évaluée par les indicateurs comportementaux (94,73%), la douleur animale n'est généralement pas quantifiée. Les analgésiques les plus utilisés étaient les glucocorticoïdes (*dexaméthasone*, prednisone) et les anti-inflammatoires non stéroïdiens (*phénylbutazone*, acide tolfénamique et méloxicam). Le pourcentage d'animaux recevant des analgésiques après l'opération était de 88,99% chez les chiens et de 79,70% chez les chats. Peu de patients (28,72%) ont été pesés avant l'anesthésie. Les paramètres les plus fréquemment

surveillés sont les fréquences cardiaque (78,95%) et respiratoires (73,68%). L'utilisation de l'anesthésie et le choix des molécules analgésiques varient significativement d'une ville à l'autre (p = 0,00), et des chiens aux chats (p = 0,03). L'acépromazine était le prémédicant le plus couramment utilisé chez les chiens et les chats. La kétamine était l'agent d'induction anesthésique le plus fréquemment utilisé chez les chiens et les chats. Les complications rencontrées par les vétérinaires lors de l'anesthésie étaient le réveil pendant l'anesthésie et l'arrêt cardiorespiratoire. Le taux de mortalité anesthésique était de 0,63%. Cette étude suggère qu'un soin particulier soit observé, en particulier lors des examens préanesthésiques et l'utilisation périopératoire des médicaments (analgésique et anesthésique) afin réduire l'incidence des complications.

Mots-clés : Douleur, anesthésie, analgésie, enquête, chats, chiens, surveillance, Cameroun.

Introduction

Pain is an inherent complication of any surgical procedure that generates major consequences in terms of animal welfare and health. Poor management of operative pain in animals degrades their general state, hinders their rehabilitation, hampers their recovery , makes them aggressive and depressive, all of thesed contribute to intensifying the necessity and duration of post-operative care (Levionnois, 2015). *Anaesthesia* is now an integral part of the practice of companion animal medicine (Hubbell, 2006), as it places the animal in optimal conditions for carrying out the intervention, in particular by limiting its stress and perception of the pain (Junot and Touzot-Jourde, 2015). Studies on anaesthetic monitoring have highlighted the use of acepromazine and *xylazine* as the best premedicants (Wagner and Hellyer, 2002; Joubert, 2006; Pestean *et al.,* 2016). Thiopental was the most widely used anaesthetic induction molecule in several countries (Clarke and Hall, 1990; Nicholson and Watson, 2001; Joubert, 2006; Bille *et al.,* 2012). The most frequent anaesthetic complications were apnea (Clarke and Hall, 1990; Farges, 2012), hypotension (Gaynor *et al.,* 1999) and awakening during *anaesthesia* (Pestean *et al.,* 2016). Many veterinarians agreed on the urgent need for effective management of operative pain in dogs and cats (Perret-Gentil *et al.,* 2014; Beswick *et al.,* 2016), requiring the same considerations as those compared with analgesic treatment (Lorena *et al.,* 2014). Several studies have shown that the proportion of animals receiving peri and/or post-operative analgesics increases significantly over time; opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) being the most prescribed (Dohoo and Dohoo, 1996; Lascelles *et al.,* 1999; Williams *et al.,* 2005; Hewson *et al.,* 2006; Joubert, 2006; Hunt *et al.,* 2015; Pestean *et al.,* 2016). However, the fear of the side effects of poorly known therapeutics, the difficulty of identifying and quantifying an analgesic condition, the cost of analgesics and the supply difficulties for certain substances subject to legislation are still constraints to the implementation of an effective analgesia, particularly in Brazil and France (Hugonnard *et al.,* 2004; Bille, 2008; Lorena *et al.,* 2014; Bruyas, 2015).

To our knowledge, there is no information on the status of Cameroon veterinarians attitudes to the provision of perioperative analgesics and routine anaesthetic protocol used in small animals. The present survey was undertaken to assess the current attitudes of Cameroon veterinarians regarding the evaluation and treatment of perioperative pain and routine anaesthetic management in dogs and cats.

Materials and Methods

This study was carried out in the cities of Douala and Yaounde, which represents 53% of private veterinarians in Cameroon. A questionnaire conceived using Sphinx plus® software, divided into five parts with a total of 63 questions was presented to 23 veterinarians from January to May 2017. The questionnaires were based on previously published studies conducted in small animals (Hugonnard *et al.,* 2004; Farges, 2012; Bruyas, 2015). Each participant was assured that the answers would remain anonymous. Almost all of the

questions were multiple choice, in order to optimize the exploitation of the results. The questionnaires were previously submitted to two veterinary practitioners in order to test their comprehensibility and the absence of ambiguity.

Part 1 soughted demographic information including gender, age, year of graduation, veterinary school attended and further studies (participants were asked whether they considered their knowledge in use of anaesthetic and analgesic drugs adequate, what form of further education would be most appropriate for increasing their knowledge).

Part 2 focused on pain assessment; veterinarians were asked about their level of concern in the areas of pain evaluation and relief in dogs and cats undergoing surgery. They were also asked to choose from a list of three pain indicators they considered the most effective.

Part 3 dealt more precisely with used postoperative analgesic protocols. Participants were asked specifically about their use of NSAIDs, opioids and *glucocorticoids*. The percentage of animals receiving analgesics postoperatively following 10 different categories of surgery were calculated (Dohoo and Dohoo, 1996). Five categories were the same for both dogs and cats; namely, orthopaedic surgery, *ovariohysterectomy*, castration, ovariectomy and wound healing. The remaining categories for dogs were tail amputation, aural *hematomas*, ear cropping, lumpectomy and *hematomas*.

Part 4 described the course of *anaesthesia* by identifying the technical gestures carried out from the pre-anaesthetic examination up to the awakening, the molecules and protocols most used in *anaesthesia*. Participants were asked about pre-anesthetic patient evaluation, whether or not patients were weighed, use of IV catheters, fluids, and endotracheal intubation. The induction technique used with reference to intravenous *anaesthesia*, the premedication, induction and maintenance agents commonly used for dogs and cats were requested. The induction techniques comprised of administering the intravenous anaesthetic agent. Information on the monitoring method and parameters monitored were obtained.

The last part addressed the issue of complications and incidents occurring during canine and feline *anaesthesia*. Global mortality rate was calculated from the total number of deaths reported divided by the number of anaesthetics given. The different factors susceptible of justifying their occurrence were also examined and compared to others reported in earlier studies.

The data was entered into spreadsheet (Excel 2016) and descriptive statistics were used to analyze frequency distributions. For most questions, results were expressed as either the percentage of total participants or the percentage of surgical cases performed. Statistical analyses were performed with IBM SPSS statistics® version 23. A chi-square (χ^2) test was performed using a 5% level of significance.

Results

Characterization of investigated veterinary clinics

During the study period, there were 23 practicing veterinarians in the clinics, 19 of them (36.84% in Douala and 63.15% in Yaounde) were willing to contribute to the study, with a participation rate of 82.60%. Most of the veterinarians surveyed (52.63%) reported to have graduated from school between 2001 and 2010. Very few practicing veterinarians in canine clinic in Cameroon (15.79%) received post-doctoral specialization in surgery. The personal and demographic data are presented in Table 1.

Pain indicators considered to be the most reliable were also valid for dogs and cats. Behavioral criteria (n = 18; 94.73%) were the most commonly used by clinicians to assess pain in animals, followed by lesion criteria (n = 11; 57.89%). Only four veterinarians (21.05%) use a pain intensity scale.

Perioperative analgesia

Among the veterinarians surveyed, four (21.05%) were reported to have been using pre- or per-operative analgesia, two (10.52%) used it

Table 1: Demographic data of Cameroon veterinarians participants to a survey of analgesic use and routine anaesthetic management in dogs and cats

Demographic	Gender		Total	Percentage
	Female	Male		
	8 (42.11%)	11 (57.89%)	19	
Year of graduation				
1981-1990	0	1	1	5.26
1991-2000	2	2	4	21.05
2001-2010	4	6	10	52.63
2011-2017	2	2	4	21.05
Total	8	11	19	100.00
Veterinary school attended				
Inter-State School of Sciences and Veterinary Medicine (Senegal)	3	3	6	31.58
Ahmadou bello university zaria kadouna (Nigeria)	1	2	3	15.79
Ukraine Academy of Agriculture in Kiev	1	1	2	10.53
School of Science and Veterinary Medicine (Cameroon)	2	0	2	10.53
University of Maïduguri (Nigeria)	0	2	2	10.53
Cureghem veterinary school (Belgium)	1	0	1	5.26
Agronomic and Veterinary Institute Hassan II (Morocco)	0	1	1	5.26
University of Lubumbashi (Congo)	0	1	1	5.26
University of Nigeria Nsukka	0	1	1	5.26
Age				
21-30	2	0	2	10.53
31-40	2	5	7	36.84
41-50	2	4	6	31.58
51-60	2	2	4	21.05
Clinic's status				
Responsible	5	9	14	73.68
Employed	1	2	3	15.79
trainee	2	0	2	10.53
Total	8	11	19	100.00
City				
Douala	3	4	7	36.84
Yaounde	5	7	12	63.16
Total	8	11	19	100.00
Post-graduate qualifications (specialization)				
None	7	9	16	84.21
Yes	1	2	3	15.79
Total	8	11	19	100.00

Table 2: Frequencies of NSAIDs and *Glucocorticoids* used in the perioperative period in dogs and cats by Cameroon veterinarians

Analgesic	Douala		Yaounde		Total	Percentage
	Cat	Dog	Cat	Dog		
Dexamethasone	8	176	70	289	543	56.86
Phenylbutazone	9	179	9	114	311	32.57
Tolfenamic acid	3	3	8	18	32	3.35
Meloxicam	7	18	0	0	25	2.62
Calmagine	3	16	0	3	22	2.30
Diclofenac	1	5	0	0	6	0.63
Ketoprofen	2	4	0	0	6	0.63
Prednisone	0	0	1	2	3	0.31
Indomethacin	0	2	0	0	2	0.21
Betamethasone	0	1	0	0	1	0.10
Carprofen	0	1	0	0	1	0.10
Cortisone	0	0	1	0	1	0.10
Prednisolone	0	0	0	1	1	0.10
Amyl salicylate	0	1	0	0	1	0.10
Total	33	406	89	427	955	100.00

Table 3: Percentage of animals receiving postoperative analgesics following selected surgical procedures carried out by Cameroon veterinarians.

Surgery	Cats		Dogs	% Receiving postoperative analgesics	p-value
	Number of surgeries	% Receiving postoperative analgesics	Number of surgeries		
Castration	34	100.00a	61	81.97b	0.008
Tail amputation	0	0.00	32	96.88	/
Hematomas	0	0.00	39	69.23	/
Aural hematomas	1	0.00	59	69.49	/
Orthopedic surgery	8	62.50a	76	93.42b	0.005
Ear cropping	0	0.00	5	80.00	/
Ovariectomy	24	100.00a	7	85.71a	0.060
Ovariohysterectomy	15	66.67a	6	100.00a	0.100
Wound healing	27	77.78a	287	76.66a	0.895
Lumpectomy	0	0.00	24	79.17	/
Total	109	88.99a	596	79.70a	0.053

[a,b]: Difference between dogs and cats significantly different (P < 0.05)

irregularly and twelve (63.15%) did not use it at all.A veterinarian did not comment on this issue. Seventeen veterinarians (89.47%) routinely used an analgesic agent in postoperative surgery while two of them (10.52%) used it irregularly. *Glucocorticoids* (89.47%; n=17) and NSAIDs (84.21%; n=16) were the most common used analgesics in both species. The use of opioids was limited. Veterinarians practicing in Douala used more NSAIDs compared to

Yaounde [χ^2 (1, N = 932) = 68.42; p <0.05]. Conversely, veterinarians in Yaounde preferred *corticosteroids*. NSAIDs are more commonly used in dogs than cats [χ^2 (1, N = 932) = 3.90; p = 0.048]. Contrarily, *corticosteroids* were frequently used in cats (67.2% to 57.7%). The most popular *Glucocorticoids* used in dogs and cats were *Dexamethasone* (n=543, 56.56%) and *Prednisone* (n=3, 0.31%) (table2). The NSAIDs used by veterinarians were *phenylbutazone* (n=311, 32.57%), tolfenamic acid (n=32, 3.35%), *meloxicam* (n=25, 2.62%) and *ketoprofen* (n=6, 0.63%). As shown in table 3, the percentage of animals receiving post-operative analgesics was 88.99% in cats and 79.70% in dogs. The used frequency of analgesics did not vary significantly from one species to another (p = 0.053).

Anesthetic follow-up

In this study, 67.87% of patients underwent a clinical examination before the surgery. The temperature was taken in 55.80% of the animals to be operated. The additional examinations were performed in 2.37% of the cases and these represented mostly radiological examinations. Few patients in Cameroon (28.72%) are weighed before applying *anaesthesia*. Six veterinarians (31%) reported that care takers were trained to monitor each patient under *anaesthesia* while 12 (63%) were not. One of the veterinarian turndown the request to answer any of this question. Three veterinarians (15.78%) considered that their staff were sufficiently trained in reanimation, eight (42.10%) felt that they were not sufficiently trained and six (31.57%) felt that their staff weren't trained at all. The introduction of a venous catheter was carried out systematically by 35.29% of the veterinarians, 41.17% did it a times and 23.52% did not do it at all. The main difficulties encountered by veterinarians in the practice of anaesthetics are the unavailability of anaesthetic agents (36.84%), the implementation of reanimation (31.57%), endotracheal intubation (21.05%) and the calculation of anesthetic doses (15.78%) (Fig. 1). The evaluation of anaesthetic unconsciousness was preferentially performed through the ocular reflex (21.88%), the position of the eyeball (20.31%) and the muscle tone (17.19%). The most commonly-monitored parameter in this survey were heart rate (78.95%), followed by respiratory rate (73.68%) and pulse rate (47.37%) (Table 4).

Table 4: Parameters used in anaesthetic monitoring by clinician veterinarians in Cameroon

Anesthetic Surveillance Parameters	Number of respondents	Percentage
Depth of anaesthetic unconsciousness		
Eye Reflexes	14	73.68
Position of the eyeball	13	64.42
Muscle tone	11	57.89
Pupillary diameter	10	52.63
Control of Sphincters	9	47.36
Bending Reflex	5	26.31
Oropharyngeal reflexes	2	10.52
Anesthetic monitoring of vital parameters		
Cardiac frequency	15	78.95
Respiratory rate	14	73.68
Arterial pulse	9	47.37
Body temperature	8	42.11
Color of mucous membranes and surgical site	5	26.32
Arterial blood pressure	4	21.05
Capillary filling time	4	21.05

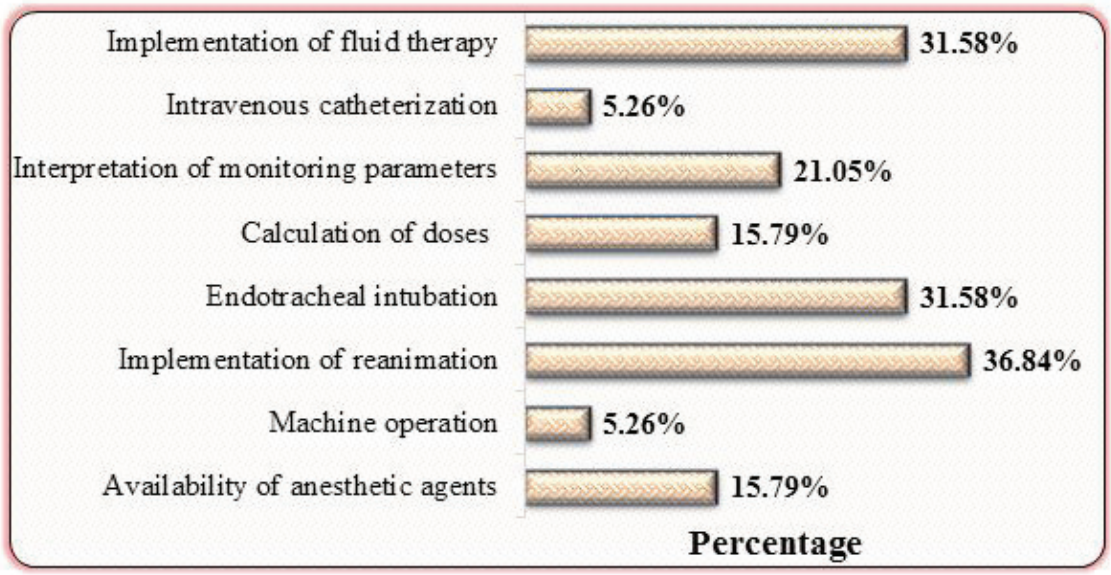

Figure. 1: The main difficulties encountered in the practice of *anaesthesia*

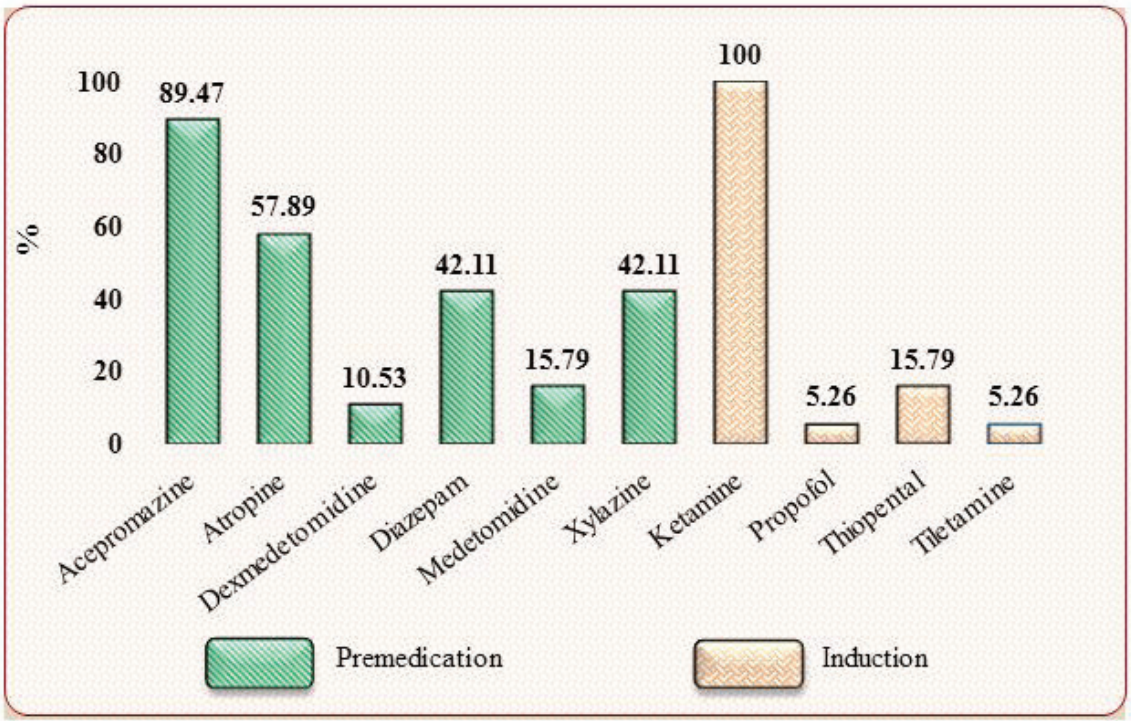

Figure 2: Premedication and anaesthetic agents used for the induction and maintenance of *anaesthesia* in dogs and cats

Almost all veterinarians (n = 18; 94%) systematically administered an anaesthetic agent before performing any surgical operation. Eighteen veterinarians (94.73%) administered a premedication separately from anaesthetic induction. The premedications administered to dogs and cats were acepromazine (89.47 %; n = 17), atropine (59.89 %; n = 11) and *xylazine* (42.11; n=8). The induction agents used for *anaesthesia* of cats and dogs were ketamine

Table 5: Most recurrent anaesthetic complications

Anesthetic incidents	City		Number of participants (%)
	Douala	Yaounde	
Awakening during *anaesthesia*	7 (36.84)	8 (42.11)	15 (78.95)
Extended wake-up	5 (26.32)	5 (26.32)	10 (52.63)
Heart failure	4 (21.05)	6 (31.58)	10 (52.63)
Hypothermia	4 (21.05)	4 (21.05)	8 (42.11)
Tachycardia	3 (15.79)	3 (15.79)	6 (31.58)
Alteration of respiratory rate or rhythm	0 (0.00)	5 (26.32)	5 (26.32)
Convulsion	2 (10.53)	3 (15.79)	5 (26.32)
Hypotension	5 (26.32)	0 (0.00)	5 (26.32)
Altering the frequency and/or heart rate	0 (0.00)	4 (21.05)	4 (21.05)
Apnea	1 (5.26)	3 (15.79)	4 (21.05)
False swallowing	2 (10.53)	2 (10.53)	4 (21.05)
Hypoventilation and its consequences	3 (15.79)	1 (5.26)	4 (21.05)
Bradycardia	2 (10.53)	1 (5.26)	3 (15.79)
Cardiac Dysrhythmias	1 (5.26)	2 (10.53)	3 (15.79)
Dyspnea	1 (5.26)	1 (5.26)	2 (10.53)
Corneal dryness	0 (0.00)	1 (5.26)	1 (5.26)

(100 %, n = 19), thiopental (15.79 %, n = 3) and propofol (5.26 %, n =1) (Fig. 2). It was observed that frequent complications that veterinarians in Cameroon encountered during *anaesthesia* were awakening during *anaesthesia* (78.95%), cardiorespiratory arrest (52.63%), prolonged recovery (52.63%) and hypothermia (42.11%) (Table 5). The mortality rate for all practices was 0.63%.

Discussion

The rate of participation in this study (82.60%) was quite high compared to previous studies (Hugonnard *et al.,* 2004; Williams *et al.,* 2005; Hewson *et al.,* 2006). This can be explained by the method of administering the questionnaire, through interviews. On the other hand, the distribution of the questionnaire in the studies mentioned above was essentially made by e-mail. Veterinarians seemed less confident in their ability to manage animal pain. Only 15.79% of practicing veterinarians received post-doctoral specialization training in surgery. Hence the overwhelming desire of 86% of these veterinarians to take part in refresher courses to improve their skills.

Almost all clinicians (94.73%) used behavioural criteria as the best indicator of pain in animals followed by physiological and lesion-related indicators. In particular, the attitude of animal and vocalization are also noted in several studies (Hugonnard *et al.,* 2004; Weber *et al.,* 2012; Bruyas, 2015). These indicators are among the most conspicuous during clinical consultation and provide information on both the sensory and emotional components of pain (Murrell, 2016). In this study, very few veterinarians (15.78%) used a quantitative grid of animal pain. Although pain grids are simple to use, offering an integrated and reasoned approach, their use is very marginal in Cameroon as in several other countries (Hugonnard *et al.,* 2004; Weber *et al.,* 2012; Bruyas, 2015).

The percentage of animals receiving post-operative analgesics was similar to those reported in Canada and Romania by Hewson et al. (2006) and by Pestean et al. (2016), respectively. It has been shown that

after surgery, the use of analgesic is strongly recommended because it facilitates the recovery of the animal (Hewson *et al.,* 2006). There was no significant difference between species in the postoperative administration of analgesics, which was also the case in Brazil (Lorena *et al.,* 2014). Concerning the respective popularity of different classes of analgesics, previous studies have shown a marked predilection for opioids especially butorphanol, buprenorphine, methadone, morphine and tramadol (Dohoo and Dohoo, 1996; Wagner and Hellyer, 2002; Williams *et al.,* 2005; Weber *et al.,* 2012; Lorena *et al.,* 2014; Perret-Gentil *et al.,* 2014). The results of this study demonstrated, however, a high application of *corticosteroids* and NSAIDs. These results are similar to those reported by Hugonnard et al. (2004) and Farges (2012) in France. Except for neurological pain, certain cancer pain and polyarthralgia, *corticosteroids* are indeed advantageously replaced for analgesia by morphinics, NSAIDs or a combination of both (Hugonnard, 2001). *Corticosteroids*, therefore, have a limited role to play in perioperative analgesia and their routine use cannot be justified (Joubert, 2001). The use of NSAIDs is explained by the fact that it is an analgesic class available in the veterinary market for long and this class is constituted of many molecules with a veterinary marketing authorization. Their action also last for long (Hugonnard *et al.,* 2004). The limited use of morphine in this study is discouraged because their remarkable effectiveness, the extent of their spectrum of activity and their flexibility of use make it an unrivalled weapon in the fight against surgical pain. The fear of side effects, the difficulty in identifying pain with certainty, the difficulty of supplying the opioids and the administrative burden imposed by their use are the reasons frequently mentioned for the non-use of morphine derivatives (Bille, 2008).

Few patients in Cameroon are weighed before *anaesthesia* as reported in South Africa by Joubert (2000) but lower than that reported in Canada by Dyson *et al.,* (1998). This situation is more conducive to modulating the risk of anaesthetic overdose, especially since the estimation of anaesthetic doses is still a difficulty for veterinarians. The major difficulty veterinary surgeons encounter in their practice of *anaesthesia* is the unavailability of anaesthetic and analgesic agents (thiopental, opioids, ketamine etc...), but Farges (2012) pointed out that the difficulty is reanimation. This situation forced clinicians to make greater use of medicines for humans. The intravenous catheter introduction is done daily in this study (76.46%) as reported by Farges (2012) and Pestean et al. (2016).

The ocular reflex and the position of the eyeball are preferably used by clinical veterinarians for the evaluation of the depth of narcosis. This result is more relevant as the most widely used molecule is ketamine. The use of the later leads to the persistence of ocular reflexes making the follow up delicate (Junot and Touzot-Jourde, 2015). In addition, the position of the eyeball and the ocular reflex are among the best indicators of anaesthetic mortality (Kennedy, 2016). Heart rate and respiratory rate are the most valued vital parameters in *anaesthesia*, in Cameroon as in South Africa (Joubert, 2000). Several anaesthetic agents are likely to cause depression of the cardiovascular and respiratory systems (Gaynor *et al.,* 1999), which are the most important causes of anaesthetic mortality (Brodbelt, 2009).

Acepromazine is the most widely used pre-medication molecule in both dogs and cats in Cameroon as in many other countries (Clarke and Hall, 1990; Dyson *et al.,* 1998; Nicholson and Watson, 2001; Wagner and Hellyer, 2002; Joubert, 2006). Although acepromazine may cause vasodilatation that may lead to per anaesthetic hypotension, its cardiovascular effects are minimal (Wagner and Hellyer, 2000; Smith and Murrell, 2016). It is likely to reduce the rate of anaesthetic complications in dogs due to its antiarrhythmic properties (Dyson *et al.,* 1998). In addition, the use of acepromazine has been associated with a low anaesthetic mortality rate in both dogs and cats (Clarke and Hall, 1990; Gaynor *et al.,* 1999; Brodbelt, 2009). However, its analgesic and muscle relaxation properties are weak (Junot and Touzot-Jourde, 2015).

Among the anaesthetic induction agents used in this study, the molecule preferred by veterinarians was ketamine. This result agrees with those reported by Wagner and Hellyer (2002), Alassane (2012), Farges (2012) and Pestean et al. (2016). Ketamine is among the available cheap injectable molecules on the market. Moreover, it is a relatively safe molecule of use since it induces few cardiovascular and respiratory depressions and represents an anaesthetic of choice for short-term interventions in animals (Farges, 2012; Junot and Touzot-Jourde, 2015). By stimulating cardiovascular function via its sympathomimetic effect, ketamine reduces the incidence of bradyarrhythmias and hypotension, thus limiting the rate of anaesthetic complications (Dyson *et al.,* 1998; Wagner and Hellyer, 2000). However, high doses and accumulation can lead to behavioural effects as excitation, hallucination and tremors (Perret-Gentil *et al.,* 2014). Finally, ketamine can be administered intramuscularly, intravenously and orally, making it easier to use in animals with poor venous access (Wagner and Hellyer, 2002). Thiopental is not commonly used in Cameroon (15.78%), yet it is by far the most widely used molecule in several countries, according to studies by Clarke and Hall (1990), Dyson et al. (1998), Joubert (2000), Nicholson and Watson (2001), Joubert (2006), Bille *et al.,* (2012). This can be explained by the obvious unavailability of the anaesthetic agents as noted by the veterinarians. If thiopental has the advantage of inducing good quality narcosis, its main disadvantages are strict intra-venous administration, risk of cardiac dysrhythmias, apnea or hypoventilation, and tissue irritation if injected perivascularly (Wagner and Hellyer, 2002).

From this study, it appears that one of the major constraints veterinarians face in their current practice is waking up during *anaesthesia*, which is similar to the results of Pestean *et al.,* (2016). In France, this complication was cited by 24.13% of veterinarians after apnea (Farges, 2012). This is not surprising because only 28.72% of the patients are weighed before the administration of an anaesthetic agent, thus a plausible underestimation of the doses of anaesthetics to be administered. The death rate is relatively higher than all previous studies on anesthetic complications, namely 0.23-0.29% (Clarke and Hall, 1990), 0.43% (Gaynor *et al.,* 1999), 0.11% (Dyson *et al.,* 1998), 1.35% (Bille *et al.,* 2012) and 0.25% (Pestean *et al.,* 2016). Current estimates are approximately 0.1-0.2% in healthy animals and 0.5-2% in diseased dogs and cats (Brodbelt, 2009). In most of these studies, anaesthetic mortality was associated with pre-existing disease, anaesthetic protocol, surgical technique, endotracheal intubation, fluid therapy or a combination of these factors (Brodbelt *et al.,* 2007; Brodbelt, 2009). In this study, preexisting diseases, lack of anaesthetic monitoring and hypersensitivity to anaesthetics are incriminated.

Conclusion

Animal pain, especially surgical pain, is a cause for concern for veterinary clinicians in Cameroon. While the supply of analgesics tends to be systematic for all types of surgery performed, improvements are nevertheless required in the quantitative evaluation of pain and the choice of analgesic molecules. Emphasis should be placed on pre-anaesthetic examination, in particular on the systematic weighing of animals and the carrying out of complementary examinations. In addition, the anaesthetics used must be more varied and adapted to the animal's health status and the complexity of the operation to be carried out.

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CURRENT PREVALENCE OF *FASCIOLOSIS* IN SMALL RUMINANTS IN MAIDUGURI SEMI-ARID ZONE, NORTHEAST NIGERIA

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Abstract

Small ruminants are an important source of animal protein and of special importance in those areas where cattle are of lesser importance. The study was conducted determined current prevalence of *fasciolosis* in sheep and goats in the semi-arid zone of northeast Nigeria. About 300 samples each from sheep and goats were collected and analysed for one year from June, 2015 to May, 2016. An overall prevalence of 9.65% and 9.15% was obtained in sheep and goats respectively. The prevalence of *fasciolosis* was higher in sheep (9.0%; CI: 8.8-16.2) as compare to goats (7.3%; CI: 4.9-10.9) during rainy season while during dry season, the prevalence was higher in goats (11.0%; CI: 7.9-15.1) than in sheep (10.3%; CI: 7.4-14.3) There was insignificant association ($p>0.05$) between the species in both seasons. Breed specific prevalence shows a significant association ($p<0.05$) among Uda (6.3%), Yankasa (4.0%) and Balami breed (0.0%) of sheep during dry season. Whereas in goats higher prevalence was observed in Sokoto Red (3.3%), (4.0%) followed by Cross Breed (2.3%), (3.7%) and least in Sahel White (1.7%), (3.4%) with statistically significant association ($p<0.05$) between infestation with *fasciolosis* and breeds of goats during both rainy and dry seasons. There was insignificant association ($p>0.05$) observed between ages of sheep and goats in both season. Sex specific prevalence shows a significant association ($p<0.05$) in sheep during dry season. *Fasciola* infestation was prevalent in sheep and goats in the study area. We recommend early treatment with anthelmintic and also destruction of intermediate host in order to obtained maximum benefit from small ruminants in semi-arid zone, northeastern Nigeria.

Keywords: *Fasciolosis*, prevalence, semi-arid zone, small ruminants

PRÉVALENCE ACTUELLE DE LA FASCIOLOSE CHEZ LES PETITS RUMINANTS DE LA ZONE SEMI-ARIDE DE MAIDUGURI DANS LE NORD-EST DU NIGERIA

Resume

Les petits ruminants sont une source importante de protéines animales et revêtent une importance particulière dans les zones où les bovins ont moins d'importance. L'étude a été menée pour déterminer la prévalence actuelle de la fasciolose chez les ovins et les caprins de la zone semi-aride dans le nord-est du Nigeria. Près de 300 échantillons provenant d'ovins et de caprins ont été collectés et analysés pendant un an, de juin 2015 à mai 2016. Une prévalence globale de 9,65% et 9,15% a été enregistrée respectivement chez les ovins et les caprins. On a noté que la prévalence de la fasciolose était plus élevée chez les ovins (9,0%, IC: 8,8-16,2) par rapport aux caprins (7,3%; IC: 4,9-10,9) pendant la saison des pluies, alors qu'en saison sèche elle était plus élevée chez les chèvres (11,0%; CI: 7,9-15,1) par rapport aux moutons (10,3%; IC: 7,4-14,3). On a relevé une association insignifiante ($p> 0,05$) entre les espèces au cours des deux saisons. La prévalence spécifique à la race montre une association significative ($p <0,05$) chez les races Uda (6,3%), Yankasa (4,0%) et Balami (0,0%) des moutons pendant la saison sèche. Par contre, chez les chèvres, une prévalence plus élevée a été observée dans la race Sokoto rouge (3,3%), (4,0%), suivie de la race croisée (2,3%), (3,7%), et faible chez la race Sahel blanc (1,7%) (3,4%), avec une association statistiquement significative ($p <0,05$) entre l'infestation par la fasciolose et les races de chèvres pendant les saisons pluvieuses et sèches. On a noté une association insignifiante ($p> 0,05$) entre les âges des moutons et des

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chèvres au cours des deux saisons. La prévalence spécifique au sexe montre une association significative (p <0,05) chez les moutons pendant la saison sèche. L'infestation par Fasciola était répandue chez les moutons et les chèvres de la zone d'étude. Nous recommandons un traitement précoce aux antihelminthiques ainsi que la destruction de l'hôte intermédiaire afin de tirer un bénéfice maximal des petits ruminants dans la zone semi-aride du nord-est du Nigeria.

Mots-clés : fasciolose, prévalence, zone semi-aride, petits ruminants

Introduction

Small ruminants are very important in human nutrition and in both urban and rural economies and have the potential of serving as tools for poverty reduction in Nigeria. Sheep and goats are an important source of animal protein and of special importance in those areas where cattle are of lesser importance (Nawathe *et al.,* 1985). Small ruminants are also important source of income and employment for many people globally especially in rural areas of developing countries and are raised for the production of milk, meat, leather, hair, wool and manure (Akhtar *et al.,* 2012; Raza *et al.,* 2014).

Medium and large scale farmers in commercial small ruminant farming in Nigeria managed their farms either in intensive or semi intensive conditions. This system of rearing inherently incurs different diseases which in turn reduces profitability of farming by treatment costs, reducing productivity and by mortality (Fabiyi and Lawal, 2012). Parasitic diseases, coupled with inadequate management hamper the productive husbandry of these animals (Fikru *et al.,* 2006). Disease due to parasitic infections has a great impact on worldwide economy by impeding the productivity of the small ruminants especially in developing countries. Among several parasitic infections, *fasciolosis* due to *Fasciola hepatica* and *Fasciola gigantica* is known to have significant economic impact (Henok and Mekonnen, 2011). Both F. hepatica and *F. gigantica* are transmitted by the snails of the family Lymnaesidae. Infestation with fasilolosis is usually associated with grazing in wet lands and drinking from the snail infesting watering places (Payne, 1990).

Fasciolosis occurs worldwide in acute, sub- acute and chronic forms. Large number of young flukes causes acute swelling and congestion of the liver producing an acute paranchymatous hepatitis in which the serous capsule of the liver may be sprinkled with hemorrhages and covered with fibre. In chronic *Fasciolosis* of sheep and goats, the liver becomes irregularly lobulated and distorted, but the bile ducts become thickened dilated, distended, and of bluish colour (Gracey *et al.,* 1999). All the species of *fasciola* causes morbidity, mortality in small ruminants and are associated with weight loss, anaemia and hypoproteinaemia (Horoloder and Haq, 1997). Clinically *Fascioliasis* is manifested by dullness, lack of appetite, weakness and oedematous distension of mucous surface of conjunctiva and pain on pressure exerted over liver in acute phase of infection. *Fascioliasis* affected animals show submandibular oedema, diarrhoea and shedding of wool especially in sheep. Gross pathological lesions included swollen friable liver with congestion and affected liver is covered with fibrinous exudates (Khan *et al.,* 2015). Liver fluke infection in lambs and kids is characterized by anemia, edema, weight loss and death (Yilma and Malone, 1998; Kassaye, 2011).

Despite the presence of large number of small ruminants and suitable environment for the parasite in the study area, there is no precise report on *fasciolosis* of small ruminants in northeastern Nigeria. The present study was conducted to find out the current prevalence of *fasciolosis* in sheep and goats in Maiduguri arid zone of northeast Nigeria and suggest possible ways of its control and prevention.

Materials and Methods

Study Area

The study was conducted in Maiduguri abattoir, a city situated between 11° 32'N and 11° 40'N and longitudes 13° 32'E, at an altitude

of 354m above sea level and located in the Sahel Savannahh vegetation zone, which consists of the sub-desert lands and the transition zones between the true desert (Sahara) and the Sahel with rainfall under 700 mm. The rainy season lasts for a short time (3-4 months) from June to September. This is followed by a prolonged dry season from October – May (Udoh, 1981). The small ruminant population within the state are: sheep (9,900,000) and goats (15,720,000) and the following approximate number: sheep (80 – 100) and goats (90 – 110) are slaughtered in the abattoir daily.

Sample Collection

Convenient sampling technique was used for the sampling and a total of 600 samples from sheep (n=300) and goats (n=300) were collected covering two seasons from June to October, 2015 marking the rainy season and November to May, 2016 marking the dry season. About 2g of faecal sample was collected directly from the rectum of each animal using hand (manually) with disposable hand gloves into a sample bottle for analysis. For bile collection, the whole gallbladder was removed from each animal species through gentle excision from the liver using scalpel blade. Care was taken to prevent spilling of the bile from the gallbladder to the thoracic cavity of the slaughtered ruminant. The bile was then emptied into a suitable container and 10% formalin solution was added for preservation prior to laboratory analysis. The adult liver flukes used in this study were collected from naturally infected ruminants which were brought to the abattoirs, by incising the liver along the biliary tracts taking all necessary precautions to avoid any damage to the parasite. The infected livers were squeezed manually to macerate the parenchyma and the flukes were carefully removed and placed into sterile sample bottles containing formalin and transported to laboratory for analysis.

Faecal, bile and adult parasites samples collected were placed into a clean and sterile universal bottles containing 10% formaldehyde solution for preservation and were taken to the University of Maiduguri Veterinary Parasitology Laboratory in a cool box for analysis and identification.

Laboratory Analysis

Two grams of faecal sample was weighed and grounded with pestle and mortar and mixed with 10% formal saline to make a solution. The mixture was sieved to separate it from the debris. Diethyl ether was added to the sieved mixture about one third of the test tube and centrifuged at 1500rpm for 5minute. Four layers where observed which are: diethyl ether, faecal debris, 10% formal saline and the sediment at the bottom of the test tube. The sediment was picked using pipette and was dropped on a clean slide and covered with cover slip which was observed under a microscope using low magnification for egg identification.

Bile was then centrifuged at 1500rpm for 5minutes. The sediment was picked with a pipette, dropped on a clean glass slid, covered with a cover slip and observed under a microscope using low magnification for eggs identification

Each of the adult Parasites (*Fasciola*) was washed thoroughly 2 to 3 times in a 0.9% normal saline solution to remove debris. The adult parasites were pressed between two glass slides to facilitate visualization of the internal structures and fixed in 70% methanol (Langeron, 1949). The parasites were stained with Borexcamine and mounted on slides using DPX to dry for 24 to 48 hours and observed under stereo microscope for morphological identification for speciation.

Statistical Analyses

Data obtained from the study were analyzed using descriptive statistics, Chi-square and odd ratios with JMP version 11 software (SAS Institute Inc., Cary, Nc). Analysis was considered significant at $p<0.05$.

Results

An overall prevalence of 9.65% and 9.15% was obtained in sheep and goats respectively as shown in Table 1. The prevalence

of *fasciolosis* is higher in sheep (9.0%; CI: 8.8-16.2) as compare to goats (7.3%; CI: 4.9-10.9) during the rainy season and higher in goats (11.0%; CI: 7.9-15.1) than in sheep (10.3%; CI: 7.4-14.3) during dry season, although there is no statistical significant association ($p>0.05$) between the species in both seasons as shown in Table I. The seasonal prevalence of *fasciolosis* in slaughtered sheep in relation to breeds in Maiduguri abattoir revealed no significant statistical association ($p>0.05$) between infestation with *fasciolosis* and breeds of sheep slaughtered in Maiduguri abattoir during the rainy season although high prevalence was recorded in Yankasa breed (4.3%) followed by Uda (3.7%) and least in Balami breed (1.0%). Whereas, during the dry season, highest prevalence was observed in Uda (6.3%), then in Yankasa (4.0%) and no prevalence recorded in Balami breed (0.0%) with statistical significant association ($p<0.05$) between the infestation with *fasciolosis* and breeds of sheep slaughtered in Maiduguri abattoir.

Seasonal prevalence of *fasciolosis* in slaughtered Goats in relation to breeds is also shown in Table I. The analysis revealed high prevalence in Sokoto Red (3.3%), (4.0%) followed by Cross Breed (2.3%), (3.7%) and least in Sahel White (1.7%), (3.4%)) during rainy and dry seasons respectively. There was significant statistical association ($p<0.05$) between infestation with *fasciolosis* and breeds of Goats slaughtered in Maiduguri abattoir during both rainy and dry seasons.

The comparative seasonal association of infestation with *Fasciola* in breeds of sheep and goats slaughtered in Maiduguri Abattoir is shown in Table II. The odd of the likelihood of infestation with *fasciolosis* among breeds of sheep during the rainy season was higher in the Balami (2.524) than in the Uda (0.396), higher in the Yankasa (1.571) than in the Uda (0.636) and also higher in the Balami (1.606) than in the Yankasa (0.218). There was no significant association ($p>0.05$) between the breeds of sheep in rainy season. During the dry season, the odd of the likelihood of infestation with *fasciolosis* was higher in the Balami (15.145) than in the Uda (0.066) and higher in the Yankasa (3.304) than in the Uda (0.303) with significant association ($p<0.05$). The odd of the likelihood of infestation was higher in the Balami (4.583) than in the Yankasa (0.218) with no significant association ($p>0.05$).

The likelihood of infestation with *fasciolosis* in breeds of goats during the rainy season was higher in the Sahel White (3.4) than in the Cross Breed (0.294); Sokoto Red (4.55) than in the Cross Breed (0.219) and Sokoto Red (1.338) than in the Sahel White (0.747) and the association among the breeds was not significant ($p>0.05$). Whereas during the dry season, there was significant association in the odd of the likelihood of infestation with *fasciolosis* between Sahel White (2.89) and Cross Breed (0.35); and also among Sokoto Red (6.87) and the Cross Breed (0.0337). But no significant association ($p>0.05$) was observed in the likelihood of infestation between Sokoto Red (2.38) and Sahel White (0.42).

The seasonal prevalence of *fasciolosis* in slaughtered sheep and goats in relation to age in Maiduguri abattoir is shown in Table III. The analysis revealed no significant statistical association ($p>0.05$) between infestation with *fasciolosis* and age of sheep slaughtered in Maiduguri abattoir ($\chi^2 = 0.007$, df =1, $p = 0.9340$). There was also no significant statistical association ($p>0.05$) between the infestation with *fasciolosis* and age of a sheep slaughtered in Maiduguri during the rainy season ($\chi^2 = 0.031$, df = 1, $p = 0.8602$).

Seasonal prevalence of *fasciolosis* in slaughtered goats in relation to age in Maiduguri abattoir revealed no significant statistical association ($p>0.05$) between infestation with *fasciolosis* and age of goats slaughtered ($\chi^2 = 0.003$,df =1, $P = 0.9530$); ($\chi^2 = 0.001$, df = 1, $P = 0.9814$)during rainy and dry seasons respectively.

The comparative seasonal association of infestation with *Fasciola* in ages of sheep and goats slaughtered in Maiduguri Abattoir is shown in Table IV. It revealed that during both rainy and dry seasons, the odd of the likelihood of infestation was higher in adult sheep (1.185), (1.125) than in the young sheep (0.844), (0.889) slaughtered in Maiduguri abattoir. No significant

Table I: Seasonal prevalence of *fasciolosis* in small ruminants slaughtered in Maiduguri Abattoir in relation to species and breeds

	Rainy Season, 2015						Dry Season, 2016				
	Number examined	No. +ve	Prevalence (%)	95% CI	χ^2	p- value	No. +ve	Prevalence (%)	95% CI	χ^2	p- value
Species											
Sheep	300	27	9.0	(8.8-16.2)	3.923	0.1406	31	10.3	(7.4-14.3)	4.122	0.1273
Goats	300	22	7.3	(4.9-10.9)			33	11.0	(7.9-15.1)		
Breeds of sheep											
Yankasa	156	13	4.3	(2.6-7.3)	2.308	0.3153	12	4.0	(2.3-6.9)	19.664	0.0001*
Uda	88	11	3.7	(2.1-6.5)			19	6.3	(4.1-9.7)		
Balami	56	3	1.0	(3.0-2.9)			0	0.0	(0.0-1.3)		
Breeds of goats											
Sokoto Red	192	10	3.3	(1.8-6.0)	9.565	0.0084*	12	4.0	(2.3-6.9)	19.888	0.0001*
Sahel White	73	5	1.7	(0.9-3.9)			10	3.4	(1.8-6.0)		
Cross Breed	35	7	2.3	(1.1-4.7)			11	3.7	(2.1-6.5)		

*= *Significant associations (p<0.05)*

Table II: Comparative Seasonal Association of Infestation with *Fasciola* in Breeds of Sheep and Goats Slaughtered in Maiduguri Abattoir

Breeds	Rainy season 2015					Dry season 2016			
	No. Examined	No. +ve	Odd ratio	95% CI	p-value	No. +ve	Odd ratio	95% CI	p-value
Sheep									
UD by BA	88/56	11/3	0.396	0.087, 1.341	0.1430	19/1	0.066	0.004, 0.334	0.0002 *
BA by UD	56/88	3/11	2.524	0.746, 11.548		1/19	15.145	2.995,276.19	
YA by UD	156/88	13/11	1.571	0.661, 3.679	0.3007	12/1	3.304	1.536, 7.369	0.0022*
UD by YA	88/156	11/13	0.636	0.272, 1.514		19/12	0.303	0.136, 0.651	
BA by YA	56/156	3/13	1.606	0.494,7.202	0.4546	1/12	4.583	0.871, 84.447	0.0771
YA by BA	156/56	13/3	0.623	0.139, 2.025		12/1	0.218	0.012, 1.148	
Goats									
CB by SW	35/73	7/5	0.294	0.081-0.997	0.0495*	11/10	0.35	0.128-0.921	0.0339*
SW by CB	73/35	5/7	3.4	1.008-12.539		10/11	2.89	1.086-7.805	
SR by CB	192/35	10/7	4.55	1.542-12.857	0.0072*	12/11	6.87	2.712-17.454	0.0001*
CB by SR	35/192	7/10	0.219	0.078-0.649		11/12	0.145	0.057-0.369	
SR by SW	192/73	10/5	1.338	0.465-3.911	0.6121	12/10	2.38	0.962-5.789	0.0604
SW by SR	73/192	5/10	0.747	0.256-2.471		10/12	0.42	0.173-1.039	

*Key: UD = Uda; BA = Balami; YA = Yankasa; CB = Cross Breed; SW = Sokoto White; SR = Sahel Red; * = significant associations (p<0.05)*

Table III: Seasonal Prevalence of *Fasciolosis* in Sheep and Goats Slaughtered in Maiduguri Abattoir relation to Age

Age	Number Examined	Rainy Season, 2015					Dry Season, 2016				
		No. +ve	Prevalence (%)	95% CI	χ^2	p-value	No. +ve	Prevalence (%)	95% CI	χ^2	p-value
Sheep											
Young	210	9	3.0	1.6-5.6	0.007	0.9340	10	3.3	1.8-6.0	0.031	0.8602
Adult	90	18	6.0	3.8-9.3			21	7.0	4.6-10.5		
Goats											
Young	104	7	2.3	1.1-4.7	0.003	0.9530	12	4.0	2.3-6.9	0.001	0.9814
Adult	196	15	5.0	3.1-8.1			21	7.0	4.6-10.5		

Table IV: Comparative Seasonal Association of Infestation with *Fasciola* in Age of Sheep and Goats Slaughtered in Maiduguri Abattoir

Age	No. Examined	Rainy season 2015				Dry season 2015			
		No.+ve	Odd ratio	95% CI	p-value	No.+ve	Odd ratio	95% CI	p-value
Sheep									
Y by A	90/210	9/18	0.844	0.372, 2.041	0.6946	10/21	0.889	0.409, 2.049	0.7734
A by Y	210/90	18/9	1.185	0.489,2.688		21/10	1.125	0.488, 2.443	
Goats									
Y by A	104/196	7/15	0.92	0.439-2.006	0.8287	12/21	0.745	0.362-1.588	0.4365
A by Y	196/104	15/7	1.087	0.498-2.276		21/12	1.342	0.629-2.764	

Key:Y = Young;A = Adult

Table V: Seasonal Prevalence of *Fasciolosis* in Sheep and Goats in relation to Sex Slaughtered in Maiduguri Abattoir

Age	Number Examined	Rainy Season, 2015					Dry Season, 2016				
		No. +ve	Prevalence (%)	95% CI	χ^2	p-value	No. +ve	Prevalence (%)	95% CI	χ^2	p-value
Sheep											
Female	215	16	5.3	3.3-8.5	1.628	0.202	15	5.0	3.1-8.1	7.993	0.0047
Male	85	11	3.7	2.1-6.5			16	5.3	3.3-8.5		
Goats											
Female	206	14	4.7	2.8-7.7	0.353	0.552	22	7.3	4.9-10.9	0.0084	0.7721
Male	94	8	2.7	1.4-5.2			13	4.3	2.6-7.3		

Table VI: Comparative Seasonal Association of Infestation with *Fasciola* in Sex of Sheep and Goats Slaughtered in Maiduguri Abattoir

Sex	No. Examined	Rainy season 2015				Dry season 2015			
		No.+ve	Odd ratio	95% CI	p-value	No.+ve	Odd ratio	95% CI	p-value
Sheep									
M by F	85/215	11/16	0.541	0.242, 1.249	0.1462	16/15	0.745	0.362, 1.588	0.4365
F by M	215/85	16/11	1.849	0.801, 4.135		15/16	1.342	0.629,2.764	
Goats									
M by F	94/206	8/14	0.784	0.323-2.027	0.6017	13/22	0.745	0.362-1.588	0.4365
F by M	206/94	14/8	1.276	0.493-3.093		22/13	1.342	0.629-2.764	

M = Male; F = Female

association ($p>0.05$) was found between the infestations with *fasciolosis* and age of sheep.

In the case of goats, the odd of the likelihood of infestation with *fasciolosis* during both rainy and dry seasons was higher in adults Goats (1.087), (1.342) than in the young Goats (0.92), (0.745) slaughtered in Maiduguri abattoir with no significant association ($p>0.05$) between the seasons.

TableV showed the seasonal prevalence of *fasciolosis* in slaughtered sheep and goats in relation to sex in Maiduguri abattoir. The analysis revealed that there was no significant statistical association ($p>0.05$) between infestation with *fasciolosis* and sex of sheep slaughtered in Maiduguri abattoir during the rainy season ($\chi^2 = 1.628$, df =1, $p = 0.2020$). While during the dry season, there was significant statistical association ($p<0.05$) between the infestation with *fasciolosis* and sex of a sheep slaughtered during in Maiduguri abattoir ($\chi^2 = 7.993$, df = 1, $p = 0.0047$).

The analysis also revealed no significant statistical association ($p>0.05$) between infestation with *fasciolosis* and sex of goats slaughtered in Maiduguri abattoir both during the rainy season ($\chi^2 = 0.353$, df =1, P = 0.5522) and dry season ($\chi^2 = 0.084$, df = 1, P = 0.7721).

The comparative seasonal association of infestation with *Fasciola* in relation to sex of sheep and goats slaughtered in Maiduguri Abattoir is shown in Table VI. The odd of the likelihood of infestation with *fasciolosis* during the rainy and dry season respectively was higher in female sheep (1.849), (1.342) than in the male sheep (0.541), (0.745) slaughtered in Maiduguri abattoir. No significant association was found between the infestations with *fasciolosis* and sex of sheep ($p>0.05$) in both seasons.

In goats the odd of the likelihood of infestation with *fasciolosis* during the rainy and dry seasons was respectively higher in female Goats (1.276), (1.345) than in the male Goats (0.784), (0.745) slaughtered in Maiduguri abattoir with no significant association ($p>0.05$) in the seasons.

Discussion

The prevalence of *Fasciolosis* in sheep and goats were 9.65% and 9.15% respectively was obtained in the study area. This was higher than the earlier report of Mbaya et al. (2010) and that of Ardo and Aliyara, (2014). We attributed this to the fact that the authors carry out a cross sectional study involving abattoir records and also shows that the prevalence of *fasciolosis* is in an increased in the study area. The prevalence rate in this study was higher in sheep than goats in the rainy season is in agreement with the report of (Mbaya *et al.,* 2010; Ardo and Aliyara, 2014). This is because sheep are more prone to the infection since they graze more often with cattle in areas with germinating pasture along river banks, flood plains and during rainy season where contact with metacercaria encysted grass blades is common as compare to goats who roam around human dwellings scavenging for shrubs.

In sheep and goats, the prevalence of *Fasciola* among breeds was not significant. All breeds were susceptible to *Fasciola* when exposed to the disease. The only predisposing factor to the disease among breeds of sheep was the state of their body condition. Thus, the healthy ones were more resistant to infestations by *Fasciola* than the debilitated ones. In contrast to the current study, (Preston and Allonby, 1979) found significant breed differences in susceptibility to helminths infection. They explained that the breed differences could be due to difference in resistance to parasitic infection, because some breeds have better resistance than others. This was in agreement with the current study, which recorded significant difference between *Fasciola* infestations with breeds of goats in the study area. Sokoto Red was more prevalent to *Fasciola* infestation than Cross breed. Sahel White was recorded the lowest. This could be due to acquired immunity against the disease in the study area as it is an indigenous breed.

Relationship between age and *fasciolosis* in goats indicated that the parasite had significantly higher prevalence in the young than in adult goats. The likely explanation for

the lower prevalence in the adult compared to the young age groups could be due to the supposed self-cure phenomenon (Fryod, 1969; Assanji, 1988) and high acquired immunity that increases with age. It has been reported that host may recover from parasitic infection with increasing age thereby become resistant (Winkler, 1982). Contrary to the reports of (Kamani *et al.,* 2007; Mbaya *et al.,* 2010; Ardo and Aliyara, 2014) who reported that adult sheep and goats were more infested than the young ones. This is in contrast with the current study, thus both adult and young sheep and goats were equally susceptible to the disease. This could be due to the fact that both age groups graze together in Maiduguri.

Among sheep, prevalence of *fasciolosis* was higher during the dry season compared to rainy season as reported by (Ademola, 2003). This is in agreement with the present study but in contrast to the finding of (Mbaya *et al.,* 2010). He reported higher prevalence in Maiduguri during the rainy season than in the dry season. Ardo and Aliyara (2014) and Kamani *et al.,* (2007) also gave similar report. These reports were in contrast to the findings of the current study as there were significant differences in prevalence between infestations with *Fasciola* in goats during the dry season. This could be due to their grazing habit around river banks, Lakes and streams during the dry season in search for green pasture (Queshi *et al.,* 2012).

It was observed by (Ardo and Aliyara, 2014) that female sheep and goats were more prevalent to *Fasciola* infestation than males. Soulsby (1982) and Ibrahim *et al.,* (2002) reveals that female ruminants have increase susceptibility to *fasciolosis* due to hormonal activity during pregnancy. The above findings were in consonants with the findings of the current study which revealed significant association between infestation and sex of goats in Maiduguri arid zone.

To the best of our knowledge, this is the current study on *fasciolosis* in small ruminants in the semi-arid zone of northeast Nigeria. *Fasciola* infestation was prevalent in sheep and goats seasonally and was endemic in Maiduguri arid zone since the prevalence of the disease was high in both seasons with an increase during the dry season. During the dry season, Uda and Sokoto red breeds of sheep and goats respectively were more prevalent to *fasciolosis*. Among sexes, female sheep were more prevalent than the male. All ages of sheep and goats were equally infested in both rainy and dry seasons respectively. Prevention and control measures should be taken seriously by early treatment with anthelmintic and also destruction of intermediate host in order to obtained maximum benefit from small ruminants.

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Conflict of interest:

The authors declare that they have no conflict of interest.

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REPRODUCTIVE PERFORMANCE OF SINGLE AND DOUBLE ARTIFICIAL INSEMINATION PROTOCOL IN SWINE

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Abstract

The aim of the research was to contribute towards improved pig reproductive performance in Uganda, through determining community boar stud semen quality as affected by boar traits and comparing the performance of single and double insemination. Semen ejaculates (n=36) from mature boars (3Large white, 3 Camborough) were studied for quality in relation to breed, boar age and individual boar. In addition, single and double inseminations were carried out during in vivo fertility trials (n = 84). The data was analyzed for correlation using Spearman rank-order correlations and variance of means by Mann-Whitney test of SPSS version 20. There was a significant positive correlation between boar age and semen volume ($rs = 0.849$, $p<0.05$), semen density ($rs\ (36) = 0.709$, $p<0.05$), total sperms ($rs\ (36) = 0.798$, $p< 0.05$), motility ($rs\ (36) = 0.571$, $p<0.05$), membrane integrity ($rs\ (36) = 0.713$, $p<0.05$) and sperm viability ($rs(36) = 0.875$, $p<0.05$). The semen quality significantly varied ($p<0.001$) between individual boars. The sperm motility of the Camborough was significantly higher than LargeWhite ($p<0.05$). The conception rate in double insemination (94.4%) was not significantly different ($p>0.05$) from single insemination (89.6%). The mean litter size for single insemination was 8.16 ± 0.34, (range 2-13 piglets) while for double, it was 9.00±0.39, (range 4-16 piglets). There was a positive relationship between semen quality and boar age. The performance of single dose in terms of piglets per insemination was higher than in double dose. Thus, a single AI dose is as good as a double dose and should be promoted among smallholder farmers who need access to low cost but high gain from breeding services. The single dose protocol seem to reduce breeding costs, however, an investigation of cost benefit analysis is needed to establish its cost effectiveness in commercial artificial insemination.

Key words: Boar traits, insemination protocol, semen fertility (*in vitro* & in vivo)

PERFORMANCE REPRODUCTIVE DU PROTOCOLE D'INSEMINATION ARTIFICIELLE SIMPLE ET DOUBLE CHEZ LES PORCS

Resume

Le but de la recherche était de contribuer à l'amélioration de la performance reproductive des porcs en Ouganda, en déterminant la qualité du sperme de verrats communautaires tel qu'affectée par les traits des verrats et en comparant les performances des inséminations simple et double. Les éjaculats (n = 36) de verrats matures (3Grand blanc, 3 Camborough) ont été étudiés pour déterminer leur qualité sur base de la race, de l'âge du verrat et du verrat individuel. De plus, des inséminations simples et doubles ont été réalisées lors d'essais de fertilité in vivo (n = 84). Les données ont été analysées pour déterminer la corrélation en utilisant les corrélations de rang-ordre de Spearman et la variance des moyennes par test de Mann-Whitney de SPSS version 20. On a noté une corrélation positive significative entre l'âge du verrat et le volume du sperme ($rs = 0.849$, $p <0.05$), la densité du sperme ($rs\ (36) = 0,709$, $p <0,05$), le nombre

total de spermatozoïdes (rs (36) = 0,798, p <0,05), la motilité (rs (36) = 0,571, p <0,05), l'intégrité de la membrane (rs (36) = 0,713, p <0,05) et la viabilité des spermatozoïdes (rs (36) = 0,875, p <0,05). On a noté une variabilité significative (p <0,001) de la qualité du sperme entre verrats individuels. La motilité des spermatozoïdes du Camborough était significativement plus élevée que celle de LargeWhite (p <0,05). Le taux de conception en double insémination (94,4%) n'était pas significativement différent (p> 0,05) de celui de l'insémination unique (89,6%). La taille moyenne des portées pour une insémination unique était de 8,16 ± 0,34 (fourchette de 2 à 13 porcelets) alors que pour la double insémination elle était de 9,00 ± 0,39 (fourchette de 4 à 16 porcelets). On a relevé une relation positive entre la qualité du sperme et l'âge du verrat. La performance d'une dose unique en termes de porcelets par insémination était plus élevée qu'en double dose. Ainsi, une seule dose d'IA est aussi bonne qu'une double dose et devrait être encouragée chez les petits exploitants qui ont besoin d'accès à un coût faible mais à un gain élevé des services de sélection. Le protocole de dose unique semble réduire les coûts de reproduction, cependant, une étude de l'analyse coûts-avantages est nécessaire pour établir son rapport coût-efficacité dans l'insémination artificielle commerciale.

Mots-clés : traits du verrat, protocole d'insémination, fertilité du sperme (in vitro & in vivo)

Introduction

In 2011 the Food and Agricultural Organization (FAO) warned that by 2050 an expanded world population will be consuming two thirds more animal protein than it did then, bringing new strains to bear on the planet's natural resources (FAO, 2011). However, 44% of world meat protein consumption is derived from pork and pork products (FAO, 2001). It has been observed that pork consumption has risen tenfold whereas beef is on the decline (UBOS, 2009; FAO, 2011). This is why pig business is said to play a central role among urban and peri-urban farmers in many developing countries (Kugonza et al, 2015). In Africa, Uganda is the leading pork consuming country with a consumption rate of 3.4 kilograms per person per year (Nabikyu and Kugonza 2016). The industry in Uganda plays an important role in improving the standard of living by creating employment opportunities, providing a source of food and generating income (Ikanni and Dafwang 1995). In an era, with high poverty levels and increasing population (UBOS, 2014 ; MFPED, 2015) and projected doubling in the world levels of animal protein consumption by 2050 (FAO, 2011), promotion of efficient productivity of prolific sources of animal proteins like pigs, offers an excellent solution and a momentous strategy. Despite the potential of the pig to reduce poverty and increase nutritional security, pig productivity remains low.

This has majorly been attributed to inefficiency in pig production and reproduction (Hodson, 1980; Pekwort, 2001). Reproductive performance of the sow herd is the key factor, if not the major factor, in controlling the efficiency of swine production (Hodson, 1980; Pekwort, 2001). It has indeed been demonstrated that profitability in any commercial livestock-breeding unit is related closely to reproductive efficiency (Althouse, 2015), which is influenced by choice of breeds and the structure of the breeding programs (Johnson, 1980).

FAO (2011) points out that over the last 40 years the surge in livestock production resulted largely from an increase in the overall number of animals being raised. But "it is hard to envisage meeting projected demand by keeping twice as many poultry, 80 percent more small ruminants, 50 percent more cattle and 40 percent more pigs, using the same level of natural resources as currently, "Rather, increases in production will need to come from improvements in the efficiency of livestock systems in converting natural resources into food and reducing waste.

Livestock genetic resources (sperms and ova) are the most important capital for livestock propagation and their efficient utilization ought to be part of the overall efficiency equation. A number of reproductive biotechnologies like artificial insemination and Embryo transfer have been reported to

conserve desired breeds as well as improving production and productivity in livestock (Cavalieri *et al.,* 2004).

By the end of the 1990s, close to 50% of the worldwide gilts and sows were inseminated (Roca *et al.,* 2006). By 2006, European countries, like Belgium, Italy, the Netherlands, Norway and Spain, had more than 80% of sows and gilts being bred by AI and in North America and Brazil the percentage had reached 75% (Roca *et al.,*2006). Despite being widely practiced elsewhere, AI in pigs had not been successfully implemented in Uganda until the year 2011 when the College of Agricultural and Environmental Sciences (CAES) of Makerere University successfully helped a pig farmer in Kira Town council to produce twelve (12) piglets using AI technology (Mutetikka *et al.,* 2011) in the pilot project. Preliminary results from pig AI pilot study showed that there is variation in quality of semen ejaculates among boars which might affect the prediction of boar fertility. The study also showed that the performance of AI on farms varied which might be due to the quality of boar semen and AI protocol among other factors. The current study aimed to establish the relationship between semen quality and boar traits as well as optimising single and double dose insemination to improve pig reproduction.

Materials and methods

The study area

Wakiso District where the study was conducted was selected basing on the priority its community places on pig production and the recent reports of success in Pig AI (Mutetikka *et al.,* 2011) in Wakiso. The District lies in the central region of Uganda bordering with Mpigi in the west, Luwero, Nakaseke and Kiboga districts in the North; Mukono in the East, and Kalangala district to the South, covering a total area of 2,807.7 square kilometers (Wakiso District, 2009). It lies at an approximate range of about 900 to 1340 meters above sea level. The district is characterized by isolated flat-topped hills with steep slopes, often merging abruptly into long and gentle pediments, which are usually dissected by relatively broad valleys. The district is divided into two main topographic zones, the Lake Victoria zone and the high land zone (Wakiso District, 2009). The dominant soils types are red gravely loams with occasional murram reddish brown sandy loam on red clay loam and yellowish sands with quartz grave. The climate is warm and wet with relatively high humidity. The rainfall is bi-modal with two wet seasons running from April to May and October to November. The dry months are January to February and July to August. The annual rainfall mean is 1320 mm though in many areas of the lake zone is between1750 and 2000mm .The minimum surface air temperature of the district is 11.0 degrees centigrade while the maximum is 33.3 degrees centigrade. With a total human population of 2,007,007 people Wakiso has the highest population in Uganda (UBOS, 2014). It has been observed that Although Wakiso district is highly urbanised, people do urban farming more than is usually noticed and it is practiced to produce food, to diversify and to strengthen the income sources of the households (Davinder, 2006).

In vitro fertility of community boar stud semen in relation to breed, individual boar and boar age
Boars, semen ejaculates and study design

The selected boars were stratified by breed. Three (3) Largewhite and three (3) Camborough boars were used. The age of the boars ranged between 8-32 months kept indoor and fed on boar feed containing 12% protein level for maintenance and sperm production. In addition, water was given ad libtum. For each boar, semen was collected twice a week for three weeks making a total of thirty six semen ejaculates (Table 1) which were evaluated.

Each semen ejaculate was pre-diluted at a ratio of 1:1 for motility and concentration evaluation. Final extension based on motility and concentration was made for further semen evaluation in triplicates. The boar semen was stored at 17-18oC during laboratory evaluation.

Boar semen collection methods, instruments and tools.

The gloved hand (Maes, *et al.,* 2010) was used to collect semen from trained boars. The Polyvinyl gloves (powder free) were used. The semen collection vessel was pre warmed (370C) together with semen bag (1L) and gauze to filter out the gel portion of the semen were used. Before collection the boar was stimulated (5-10 minutes) and prepucial pouch fluids emptied. As the boar mounted the dummy, the spiral end of the penis (glans penis) was grabbed and with increased firm pressure on the glans penis, ejaculation was initiated. The first part of the ejaculate which is a clear watery fluid was discarded to avoid contamination of semen ejaculate. After collection, the filter with gel was discarded, and the collection thermos container carefully covered. Once the semen sample had been collected it underwent macroexamintaion for appearance, odor, colour and volume before extension.

Semen extender and extension

The extender used was adopted from Foley et.al, (1963). The two cardinal rules for effective boar semen extension of (1) Adjusting extender temperature to within 1°C of semen temperature and (2) Always adding the extender to the semen not the other way round were followed. Thus rapid cooling of ejaculate was avoided by pre dilution of ejaculates 1:1 and letting pre dilute ejaculates stand at room temperature for 6 hours to induce cold resistance before final dilution. The semen was extended at 1:7-10 depending on the motility and concentration according to (Foley et.al, 1963). The extender was prepared and kept at 37oC for least 2 hours before extension in order to let the PH and ions achieve equilibrium. After the final dilution, filling of commercial doses (100mls semen bottles) was done and the semen allowed cooling down gradually to 17°C.

Boar semen evaluation

The semen characteristics of ejaculates were evaluated by assessing color, volume, concentration, motility, cell membrane integrity and sperm viability. The color was assessed by visual observation of each ejaculate (Rozeboom, 2000). The volume was measured by weighing the ejaculate. It is known that density of pig semen is approximately one gram per cc therefore; 1 gram is equal to 1 ml (Alfonso, 2012). The sperm concentration was determined by Hemacytometer (Knox, 2004). To get the sperm concentration, a sub-sample from the ejaculate was taken by placing 2-3 drops on a clean dry microscope slide. Then a Unopette capillary pipette was used to obtain a 10µl sample .The semen sample in the capillary tube is then added to the Unopette dilution chamber at 1:100 and mixed. The diluted semen sample was then applied to both chambers of hemacytometer. After 5minutes, the counting of sperms in both chambers was done with a light microscope, (400x magnifications) in 5 diagonal squares taking care not to count sperm heads that touched the right or bottom ripple lines of the squares. Average for the two counts was considered a single data set. The actual concentration was obtained by the formula; Sperm /cc = sperm in 5 squares x 5 x dilution rate (100) x hemacytometer chamber volume (104).

The sperm viability was assessed using Eosin and Nigrosin test (Bamba, 1988). To determine the viability of spermatozoa, a drop of the semen sample was mixed with a larger drop of the Eosin (1%) and Nigrosin (5%) stains on a pre-warmed slide with an applicator stick. A thin smear was then made with another slide. After air-drying, the smear was observed under a phase-contrast microscope (40×) for unstained heads of the spermatozoa (live or viable sperms before staining) and stained or partially stained heads of the spermatozoa (dead sperms). This was performed in three fields, the mean of the observations calculated and considered a single data point.

The sperm membrane integrity was assessed using the hypo osmotic swelling test (HOST), (Jeyendran *et al.,* 1984). The hypo osmotic solution was prepared by dissolving 0.735 g of sodium citrate and 1.351g of fructose in 100 ml of distilled water (osmotic pressure = 190 mOsm/kg). For the assay, 50 µL of semen

was mixed with 500 µL of the pre-warmed (37°C) HOST solution and incubated at 37°C for 45 min. Two slides of the sample ware then prepared and examined under a phase-contrast microscope (40×). Two hundred spermatozoa were microscopically counted, and the number of spermatozoa showing characteristic swelling of the tail, an indication of an intact plasma membrane, was recorded, percentage calculated and mean taken as a single data set.

Motility was assessed as described by Rozeboom (2000). To determine the percentage motility, a very small drop of diluted spermatozoa was placed on a warmed microscopic slide and over laid with a cover slip and viewed under a (400 X) power microscope. Then 10 cells were counted in 3 different fields and average percentages of motile cells (only those with forward motility) were obtained to determine the progressive motility.

Performance of single dose and double dose inseminations

Research design

The recruitment of farmers into the experiment was based on voluntarism and random allocation. To avoid bias in the conduct of the field trial, information about the test was masked from the farmers. Therefore a complete randomized field trial (Experimental) design was used. Having publicized the free pig AI, any pig farmer who reported a gilt or sow in estrus qualified for either single insemination AI (Cluster 1, n=48) or double insemination (Cluster 2, n=36). Farmers who called were allocated to a different cluster based on random number every after three calls. Three fertility parameters namely; pregnancy (non return to estrus in 18–25 days) indicating that the sow conceived; litter size (total number of piglets born) and sex were recorded in each cluster for statistical analysis. In single insemination, time for service was recorded from the onset of heat to determine optimal time for insemination. In double, time for first service from onset of heat and the second service was also recorded.

Field inseminations

Farmers who reported a pig in heat were probed to confirm that the gilt or sow was most probably in standing heat. This helped the researcher to decide the time to give the first and second insemination. Standing heat was ascertained by the back pressure test. The intra-cervical method (Karunakaran, *et al.,* 2013) was used in all inseminations insemination. After cleaning the lips of the vulva with tissue, the catheter was introduced by the forward and upward direction. After contact was made with the cervix, the spiral catheter was rotated anti clockwise until resistance was felt (Lock in the cervix). A clockwise turn was made in case the opening at the tip got blocked by the tissue to open. After it locked, the catheter was bent upwards and semen bottle attached. The flow of semen was largely by gravity with a slight pressure. Once insemination was completed the sow/gilt was left quiet and catheter connected for 3-5 minutes. Conception was indicated by a non return to heat after 18-22 days.

Data analysis

The data from laboratory and field trials was entered into Microsoft Excel (2007) computer software to get descriptive statistics. The cleaned data was then imported and keyed into the SPSS version 20 (IBM, 2011) for further statistical analysis. Data sets that were checked for normality using Kolmogorov-Smirnov test of normality. Not normally distributed data was transformed by log transformation. Correlation was calculated using Spearmans rank order correlation and the significance of variance of means by Mann Whittey test, t-test and Kruskal-Wallis Test. at probability level of 0.05. Linear model used in data analysis is as follows:

$$Y_{ijk} = \mu + Agei + Genotypej + individual\ boark + eijk$$

Where:

Y_{ijk} = Semen fertility of the nth boar of the ith age, of jth genotype, and of kth individual boar

µ = general mean of the semen fertility

Age_i = effect of age of boar (i = 1,2,3)

Genotype$_j$ = effect of genotype (j = 1, 2)
Individual boark = effect of individual boar (k = 1, 2, 3, 4, 5, 6)
Eijk = random error term

Results

The Mean (M + SE) values of semen quality indices.

The results for mean (M + SE) values of semen quality parameter are presented in table 2. The ejaculate volume ranged from 96 to 242 ml averaging 164.83 + 9.92 ml. The average sperm concentration was 231.47 x 10 6 + 3.750 x 10 6 sperms per ml, and ranged from 197 x 10 6 to 285 x 10 6 sperms per ml. Total sperms averaged 39.17+ 28.03 x 109 and ranged from 20.7 to59.8 billion sperms per ejaculate. The average percent progressive motility was 76.85 % + 0.51% ranging from 71.67% to 81.67%. The mean of percentage membrane intact sperms was 60.41 + 1.55 ranging from 48.67% to 73.67%. While mean percent viable sperms averaged 69.97 + 2.54 and ranged from 57.33% to 86.67%.

Semen quality under breed, individual boar and age

The semen quality values under breed, individual boars and age in months are presented in table 3. The statistical analysis of variation of semen quality indices under boar age was carried out using the Kruskal-Wallis Test. The results indicate that the variations in semen quality(indices) between the different boar age groups were statistically significant ($p< 0.05$).in volume ; χ^2 (2) = 22.550, $p < 0.05$, in semen concentration ; χ^2 (2) = 18.797, $p< 0.05$, in total sperm cells; χ^2 (2) = 21.209, $p < 0.05$, in percentage motility; χ^2(2) = 6.717, $p < 0.05$,in membrane intact sperms ; χ^2 (2) = 20.917, $p< 0.05$ and percentage viable sperms; χ^2 (2) = 24.912, $p<0.05$. The analysis for variation of semen quality indices under boar breed was carried out using the Mann-Whitney U Test. This data, indicates that the sperm motility of the Camborough group was statistically significantly higher than the large white group (U = 81.5 , p = 0.009), however the variations in the rest of the quality indices between the two breeds were not statistically significantly (U = 91, p = 0.25) for semen volume, (U = 123, p = 0.25) for semen concentration, (U = 104, p = 0.66) for total sperm cells , (U = 114, p = 0.128) for membrane intact sperms and (U = 123, P = 0.216) for sperm viability. The statistical analysis of variation of semen quality indices under individual boars was carried out using the Kruskal-Wallis Test. The results indicated that variations in semen quality

Table 1: Sample size and experimental design for in vitro fertility studies

Breed	Boars	Sample size		
		Ejaculates per week	No of weeks	ejaculates/3weeks
Large white	3	2	3	18
Camborough	3	2	3	18
Total ejaculates				36

Table 2: Mean (M + SE), values of semen quality indices

Semen quality indices	N	Mean	Min.	Max.
Volume of semen ejaculate (ml)	36	164.83 ±9.92	96	242
Sperm concentration (sperms/ml) x 10 6	36	231.47 ±3.75	197	285
Total live sperm cells x (10 9)	36	39.17± 28.03	20.70	59.80
Motile sperms (%)	36	76.85± 0.51	71.67	81.67
Membrane intact (HOST) sperms (%)	36	60.41± 1.55	48.67	73.67
Viable sperms (E+N test) (%)	36	69.97±2.54	57.33	86.67
Ph extended Semen	36	6.86 ±0.00	6.80	6.90

Table 3: Semen quality under breed, individual boar and age

Parameter	Category	N	Volume Ml	Total sperms X 10^9	Concentration sperm/ml X 10^6	Sperm Motility (%)	Intact membrane (HOST)	Viability E+N test (%)
Age (months)	12 & below	12	104.50±2.28[a]	22.52±0.40[a]	216.17±4.52[a]	75.28±0.45[a]	51.53±0.85[a]	59.28±0.35[a]
	13-24	12	176.67±16.57[b]	41.48±4.71[b]	229.00±6.09[b]	77.50±1.22[b]	61.67±2.62[b]	71.87±2.97[b]
	>24	12	224.17±3.94[c]	56.51±0.75[c]	252.50±3.34[c]	78.20±0.63[c]	69.17±0.88[c]	80.31±0.33[c]
Breed	Large white	18	143.67±13.10	33.64±3.73	228.28±5.53	75.56±0.74[a]	57.32±1.74	66.70±2.40
	Comborough	18	186.00±13.44	44.69±3.85	234.67±5.11	78.15±0.57[b]	63.50±2.40	73.24±2.39
Boar	Boar 1	6	109.17±3.62[a]	8.77±3.80[a]	207.00±4.47[a]	75.00±0.75[a]	50.06±0.35[a]	59.72±0.59[a]
	Boar 2	6	219.33±5.11[b]	53.80±1.26[b]	245.33±2.60[b]	80.84±0.37[b]	69.4±0.58[b]	80.11±1.40[b]
	Boar 3	6	229.50±4.60[c]	57.73±0.97[c]	251.67±2.47[c]	77.50±0.71[c]	71.39±0.56[c]	80.00±0.49[c]
	Boar 4	6	99.83±1.05[d]	22.50±0.588[d]	225.33±6.01[d]	74.45±0.56[d]	53.39±1.29[d]	58.95±0 .43[d]
	Boar 5	6	218.83±5.96[e]	55.29±0.96[e]	253.33 ±6.54[e]	78.89±1.03[e]	66.95±1.07[e]	80.61±0 .45[e]
	Boar 6	6	112.33±2.03[f]	23.14 ±0.30[f]	206.17 ±2.79[f]	73.34±0.86[f]	51.61±0.26[f]	60.56±0.30[f]

Means with different superscripts are spastically y different ($p<0.05$)

Table 4: Correlation between boar attributes (age, breed, individual boar) and semen fertility

	N	Semen volume	Semen concentration (sperms/ml)	Total sperms	percentage motility	Membrane integrity	Sperm viability
Boar age	36	0.849**	0.709**	0.798**	0.571**	0.713**	0.875**
Breed	36	-0.380**	-0.211	-0.310	-0.440**	-0.257	-0.209
IndividualBoar	36	-0.061	-0.043	-0.042	-0.360**	0.029	0.060

*** Correlation is significant at the 0.05 level (2-tailed).*

Table 5: Correlation between percentage motility and other semen quality parameters

	Semen volume (ml)	Semen concentration (sperms/ml)	Total sperms billions	Membrane integrity (%)	Sperm viability (%)
Sperm					
Motility (%)	0.597**	0.596**	0.588**	0.496**	0.625**
p-value	0.000	0.000	0.000	0.002	0.000
N	36	36	36	36	36

*** Correlation is significant at the 0.05 level (2-tailed).*

(indices) between the different individual boars were statistically significant ($p< 0.001$) since p-value was less than 0.05 (for semen volume; $\chi^2(5) = 29.549, p < 0.05$, semen concentration ; $\chi^2(5) = 28.320, p< 0.05$, total sperm cells; $\chi^2 (5) = 27.695, p < 0.05$, percentage motility; $\chi^2(5) = 25.057, p < 0.05$, membrane intact sperms ; $\chi^2 (5) = 31.025, p< 0.05$ and percentage viable sperms; $\chi^2 (5) = 28.474, p<0.05$)

Correlation between boar attributes (age, breed, individual boar,) and semen fertility

A series of Spearman rank-order correlations were conducted in order to determine if there were any relationships between semen volume, concentration, total sperms, membrane integrity, sperm viability and boar attributes (Table 4). A two-tailed test of significance indicated that there was a significant positive relationship between boar age and Semen volume rs (36) = 0.849, p< .05, Semen concentration rs (36) = 0.709, p< .05, Total sperms rs (36) = 0.798, p< .05, percentage motility rs (36) =0.571, p< .05, Membrane integrity rs (36) = 0.713, p< .05 and Sperm viability rs (36) = 0.875, p< .05. Therefore the higher the boar age the better was the semen quality in the study group. There was a weak negative correlation between boar breed and semen volume rest (36) = -0.380, p< .05 on one hand and sperm motility rs (36) = -0.440, p< 0.01, on the other. Similarly the correlation between individual boars and motility was significantly weak negative rest (36) = -0.360, p< 0.01 as demonstrated.

The relationship between motility and other semen fertility indices

A series of Spearman rank-order correlations were conducted in order to determine if there were any relationships between the percentage motility and other semen quality indices. A two-tailed test of significance indicated that the sperm motility correlated positively well with Semen volume rs (36) = 0.597, p<0.05, Semen concentration rs (36) =0.596, p< 0.05, Total sperms rs (36) = 0.588, p< .05, Membrane integrity rs (36) = 0.496, p< 0.05 and Sperm viability rs (36) = .625, p< 0.05 as shown in table 5.

Mean values (M + SE) for AI reproductive indices

The results for field fertility trials are presented in table 6. The overall average litter size was 8.48+ 0.26 and ranged from 2 to 16 piglets. With single AI protocol it was 8.16 ±

Table 6: Mean values (M ± SE) for AI reproductive indices

Parameter	N	Mean	Min.	Max.
Litter size (total piglets at birth) in single AI	45	8.16 ± 0.34	2.00	13.0
Litter size (total piglets at birth) in double AI	35	9.00±0.39	4.00	16.0
Female piglets at birth in single AI	45	4.13±0.21	0.00	8.00
Male piglets at birth in single AI	45	3.93±0.20	0.00	7.00
Female piglets at birth in double AI	35	4.60±0.3	1.00	10.0
Male piglets at birth in double AI	35	4.51±0.23	2.00	7.00
Time of service from onset of heat (hours) in single AI	48	24.25 ±1.17	12.5	40.0
Time of 1st service (hours) in double	36	24.31±1.52	11.5	34.7
Time of 2nd service (hours) in double AI	36	13.75±0.37	10.5	24.0
Number of inseminations per conception in single AI	48	1.15 ± 0 .05	1.00	2.00
Number of inseminations per conception in double AI	36	2.1 ±0.08	2.00	4.00

Table 7: Reproductive performance for the single and double dose breeding protocols

AI protocol	No of inseminations Per conception	Conception rate	Litter size	Sex ratio (F:M)
Single dose	1.15±0.05[a] (n=48)	89.60±4.46 (n=48)	8.07±0.33 (n=44)	1.02± 0.08
Double	2.10±0.08[a] (n=36)	94.4±3.87 (n=36)	9.06±0.40 (n=35)	1.05± 0.07

[a] *Means with different superscripts are significantly different (P<0.05)*

0.34 ranging from 2 to 13 piglets while for double AI it was 9.00 ± 0.39 and ranged from 4 to16 piglets. The overall average female piglet per litter was 4.31 ± 0.17 ranging from 0 to 9 while for males it was 4.16 ± 0.15 and ranged from 0 to 7. This varied between the two protocols. The average time lag between onsets of heat to first insemination was 24.27±0.93and ranged from 11.5 to 40 hours. The average time lag between first insemination and second insemination was 13.75±0.37 and ranged from 10.5 to 24 hours. The average number of inseminations per conception under single AI protocol was 1.15 ± 0 .05 and ranged from 1 to 2. The average for double AI protocol was 2.1 ± 0.08 but ranged 2 to 4.

Reproductive performance for the single and double dose breeding protocols

Reproductive performance was compared in terms of inseminations per conception, conception rates, litter size and sex ratio (Table 7). The difference in number of inseminations per conception between single dose and double dose was significantly different ($p < 0.05$). The conception rate was higher for double dose protocol (94.4%) than in single AI protocol (89.6%). The difference in conception rate was not significant $t(82) = -0.791$, $p = 0.431$. Similarly the mean number of piglets under double AI was higher than that of single AI protocol but not significant $t(77) = -0.729$, $p = 0.175$, ($p > 0.05$). The sex ratio between the single and double AI protocols was not significantly different $t(76) = -.217$, $p = 0.194$.

Discussion

The average ejaculate volume was (164.83 ± 9.92) and ranged from 96ml to 242ml. The normal range of boar semen volume is 100-500 ml (reference). When the volume of semen collected is below 50ml, the whole

ejaculate should be discarded. When volume is very low, it is considered inadequate because between ejaculate volume and the other characteristics there is a positive correlation (Ilinca and Cernescu, 2008). We found volume increased age and motility related to volume. The volume obtained is thus within the normal range. However, relatively lower values were reported in earlier studies by Savić *et al.,* (2013) who got a volume of 156.11ml and Hafez (1993) got 100 – 150 ml. Strzezek *et al.,* 1995 obtained a volume of 250 mls which is high the result in the study. The contrast could be attributed to several factors such as the differences in boar age, breed, frequency of collection, season and nutrition (Ilinca and Cernescu, 2008, Alfonso, 2012).

The sperm concentration results of 234.7 x 106 + 3.75 x 106 sperms per ml and ranging from 197 x 106 to 285 x 106 sperms per ml which agrees with previous reports (Turba et al 2007) who found that boar sperm was a dense ejaculate containing sperms between 0.151 and 0.400 billion spermatozoa/ml.

The motility of semen is one important attribute in predicting the potential of semen fertility. Most of the semen ejaculates had motility of 76.85+ 0.51%. Semen ejaculates with motility below 70% are though to be inferior and thus should be discarded. The progressive motility of 76.85+ 0.51% was comparable to earlier findings by Frangež *et al.,* (2005) of 70.17 to 78.04% but lower compared to 84.22% obtained by Savić *et al.,* (2013). The variation in values for motility may be attributed to the subjectivity in the visual assessment of sperm motility. The semen ejaculates had average viable sperms of 69.97 ± 2.54% varying from 57.33 to 86.67. This was low compared to 82.80±14.51 obtained by Wysokińska *et al.,* 2015 who used the eosin-gentian dye method.

The membrane intact sperms (60.41 ± 1.55) varied from 48.67 to 73.67%. This result was low compared to an average of 85.1 ± 10.7 % by Alfonso 2012 using computer assisted semen analysis (CASA). Besides the variation in methods used in the analysis, previous studies have demonstrated several factors that could have been responsible for the difference, such as nutrition, season, breed and age (Savić *et al.,* 2013). However, it was higher than that obtained in previous studies (González, et. al., 2013) who found a hypo osmotic swelling test (HOST) cells of 53.3±2.5. The variations in the osmolarity of the host solution and frequency of boar collection could be a probable explanations of these differences.

There was a difference between sperm motility between and large white. Combrough seem to have higher motility. These results suggest that the breed difference of Large white and Cambrough did not have a significant effect on ejaculate volume, concentration, total sperm cells, membrane integrity and sperm viability. The superiority in litter sizes that is reported for Cambrough breed (Okello *et al.,* 2015) could be owed to its significantly higher sperm motility. Sperm motility is also an important characteristic in predicting the fertilizing potential of an ejaculate. The reason is that a high motility is required for the sperm at the fertilization site to reach and penetrate the oocyte. A 1% increase in sperm motility in the diluted semen was correlated to an increase of 0.14 piglets per litter (Vyt *et al.,* 2008).

The results indicate that there is a significant variation in semen quality and boar age groups. Boar age positively correlated strongly with semen quality indices comparable to report by Savić *et al.,* (2013), where linear regression coefficients indicated that as the age of the boar increases, ejaculate volume and sperm motility increase. The increases in volume with increasing age has been attributed to increase in mass and size of the testes, resulting in increased production of sperm (Savić *et al.,* 2013).

There was a significant variation in semen fertility among individual boars. This agrees with earlier studies (Kommisrud *et al.,* 2002) who found a significant influence of individual boar on motility, and acrosome integrity. The individual differences in semen quality could be due to factors such as differences in age, feeding, breed, health and environment. This observation could suggest that semen doses mixed from different boars

could offer better fertility results.

The achieved average duration between onset of standing heat to first insemination was 24.27±0.93 hours while the second insemination for double AI protocol was performed at an average of 38.02±0.37 hours from the onset of heat. The AM-PM rule was observed. This time range is partially consistent with the recommendations that for double AI, gilts should be inseminated 8–12 hours after the onset of standing heat and again 12–16 hours later while sows should be inseminated 24 hr after onset of standing heat and again 18–24 hr later. (Althouse, 2015). The reasons for the partial variations from the recommended optimal timing were many but mainly lack of reliable means of transport, traffic jam, rainfall and late reporting. This calls for interventions like locating AI service providers nearer to farmers to reduce cases of sub optimal AI timing.

Conception rate of 94.4% in double was higher than 89.6% under single AI protocol. Comparably higher than results reported in the recent study by Manas *et al.,* 2014 who got 77% for double and 54% for single AI protocols. The difference could be due to several factors such as variations in insemination skills, optimal timing, semen quality and handling, nutrition, sow factors and season. However from both studies, it is clear that the performance of double AI in terms of conception rates is higher than that of single AI protocol. The most probable reason for this finding may be the highly challenging optimal AI timing involved for a Single AI protocol under field conditions. This is partly why it has been recommended that use of double insemination protocol should be adopted in farm and field conditions for better conception rate and litter size (Manas *et al.,* 2014). None the less double AI protocol may not be cost effective, since it may require double transport expenses, time, catheters, semen doses, and other related input materials and costs.

The average number of inseminations per conception under single AI protocol was 1.15±0.05, ranging from 1 to 2 inseminations. In double AI protocol, it was 2.1±0.08, ranging from 2 to 4. To reduce the number of inseminations per conception, estrous detection (heat checking) must be done carefully and without fail and the inseminations must be done correctly and at the optimal times (Jodi and Safranski, 1997).

The litter size under single AI protocol was 8.16 ± 0.34 compared to 9.00±0.39 for double AI The litter size was relatively low compared to 9 piglets in Kenya, 11.2 piglets in Ireland, 12.7 piglets in Denmark and 12.5 piglets in France (Peadar and Brendan 2007) with a potential possibility of 24 piglets that could result from ~15–24 ova released over a 1 to 4 hour period during ovulation (Althouse, 2015). The low litter size has been blamed on factors like, poor nutrition, poor breeding methods, boar factors like low fertility, gilt/sow factors like small uterine capacity, age (parity) and ovulation rates (Kirkwood and Aherne, 1985, Althouse, 2015). This suggests that to improve litter size, management, breeding methods and pig breeds ought to be improved. The revelation that the litter size under single and double AI protocols was not statistically different ($p>0.05$) suggests that on average the litter size was not influenced by the AI protocol in the study group. It implies that big litter size may be achieved with either protocol. The results agree with findings of Landblom and Nelson, (1979) in a study, who demonstrated that conception rate was improved substantially by double insemination compared to single dose, but no significant im¬provement in litter size was measured. In contrast, recent findings by Manas *et al.,* (2014) reported that the performance of double AI was better in terms of litter size (8.34) compared to (3 .20) for single AI. The differences within litter sizes could be attributed to several factors such as variations in AI timing, Sow parity, estrus detection skills and inseminators experience. The sex ratio between the single and double AI protocols was not different $t(76) = -.217$, $p = 0.194$ from the expected binomial model of 1:1. This may suggest that AI protocol does not affect the sex ratio within the litter size though some farmers claim AI produces more male than females.

The study has clearly demonstrated that single dose AI in pigs can be feasible under commercial conditions of Uganda. Insemination when the sow is in standing heat and confirming by applying back pressure (sitting on the back of a sow or gilt) has been underscored. Besides being culturally unbelievable, it was not easy to perform AI especially in gilts. The results show that the single timely AI would produce the best returns to breeding with continued optimization as double dose protocol.

Conclusion

In conclusion, semen fertility varies with boar age and individuals boar. There is a positive and high relationship between semen attributes and boar age. Sperm motility correlates positively with other semen attributes (semen volume, concentration, total sperms, membrane integrity and sperm viability). Sperm motility of comb rough breed is higher that motility of large white. Single AI and double AI protocol give relatively similar conception rates and litter size. The AI protocol as a breeding method in pigs (single dose and double dose) does not influence the sex in offspring. The study has evidently exposed facts to improve breeding in pigs. The age of the boar should be established first. Only mature boars especially above 12 months should be used in semen dose production. The sperm motility test can only be used to predict fertility of semen since it relates with the other attributes. Breed differences should be considered in semen dose production. Single dose is practical and feasible in breeding farmer's pigs, however strict AI timing is needed to achieve maximum fertility.

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Public brief

Artificial insemination although relatively new in Uganda and Africa is the best method known for breeding pigs. It is an effective method that has benefited pig farmers. In Uganda, the technology started in 2011 as a pilot project in Wakiso district and since then there have been efforts to upscale the technology to a wider community as a future breeding strategy among farmers. For example Resilient Africa Network has come up to support the innovation as regards the development of novel boar semen diluent for semen storage. Recently, National Research organization supported training of AI technicians and junior researchers to generate better knowledge about artificial pig breeding. There is still a wide gap in knowledge and therefore more training and research for pig AI development is still needed. The first inseminations in Uganda were carried out using liquid boar semen stored at 17oC and based on double dose insemination. The AI technician after some time realized that going back to serve the female in heat for the second service is costly and eventually makes the AI service be expensive. In addition, semen used was being extracted from different boars and processed into doses. However, laboratory screenings would show variation within the boars making it difficult to predict boar semen fertility. The objective of the current study was i) to determine the relationship between boar breed, individual boar and age with boar stud semen quality and ii) compare the performance of single and double insemination. The experiments based on the two objectives were set up, carried out and data collected analysed. The key findings from the study were;

i. There was a positive relationship between semen quality and boar age

ii. The semen quality among individual boars varied
iii. The sperm motility of the Camborough was higher than the LargeWhite
iv. The number of piglets born in double insemination 9.00±0.39 (range 4-16 piglets) was not different from single insemination 8.16 ± 0.34, (range 2-13 piglets).

Based on the key results above we recommend the following be supported;

i. Single dose insemination practice is possible and should be popularized as a breeding strategy since it can maintain the fertility as double dose and can reduce breeding costs. However, to maintain the same fertility, both farmers and AI technicians must be trained on Strict/optimal heat detection. Heat detection is a critical factor for the successful AI program. All pig farmers need to be trained in the basic reproductive behavior so that they are able to identify female pigs on their farms in heat and immediately inform theinseminators.
ii. Establishment of model AI centers and community based boar studs for production of high grade liquid boar semen with heritable traits that will result into faster genetic gain in pig herd communities. Enforce semen quality checks and controls at different critical control points in production based on the semen standards. Ensure that semen ejaculates must first be screened before processing to avoid inferior ejaculates.
iii. Improvement of pig breeds. Pig breeders/. breeding of better breeds are needed to provide good quality boars for semen dose production. Combo rough a newly introduced breed in Uganda shows high sperm motility compared to Largewhite. The breed can be promoted.
iv. Creation of enabling environment for enhancing of better breeding strategies for example conducive policies and support services e.g. extension services
v. Popularization of the innovation as a better breeding strategy being relatively new technology
vi. More research for development is needed since there is still wide knowledge gaps on reliable indicators of boar semen fertility, practical heat detection, semen storage e.t.c

The technology works and if promoted can revolutionarise pig farming and our economies by creating employment for youth, increase income at household through piglet sales and better nutrition, food security and creating business and trade in pork and pork products among countries. It would also stimulate other sectors of the economy for example growth of business in AI input supplies.

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EFFECTS OF PROPORTION AND ENSILING DURATION ON NUTRIENT DIGESTIBILITY AND FERMENTATION CHARACTERISTICS OF PENNISETUM PURPUREUM AND ENTEROLOBIUM CYCLOCARPUM SEEDS SILAGE USING INVITRO GAS PRODUCTION TECHNIQUE.

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Abstract

The nutritive value of Pennisetum purpureum grass with treated Enterolobium cyclocarpum seeds silage at varying proportions and at different ensiling periods was evaluated based on their chemical composition and *in vitro* fermentation. The study was a 7×2 factorial experiment with three replications. The factors were seven different proportions (100:0) as control, 85:15; 70:30) for boiled, toasted and raw *E. cyclocarpum* seeds ensiled with *P. purpureum* grass at varying proportions (85:15; 70:30 respectively) and two ensiling periods (30 and 60 days). The results revealed that crude protein (CP) contents of the silage ranged from 9.92 to 15.47 %, with 30% boiled seed+70% grass showing the highest CP contents. Silage that was ensiled for 60 days had significantly ($P<0.05$) higher CP content than in 30days period. The range of volatile fatty acids in the supernatant of the silage after *in vitro* incubation for 48h were: acetate (0.74 to 1.90 %); propionate (0.49 to 1.26 %) and butyrate (0.07 to 0.19 %). *In vitro* digestibility of silage ranged between60.33 and 81.67 % for dry matter, 58.12 and 84.98 % for crude proteinand neutral detergent fibre between68.17 and 88.13 %. Silage with 30% raw seeds + 70% grass had significantly ($p>0.05$) higher total anaerobic bacteria counts. Ensiling of the seeds and grass at 60 days produced higher total anaerobic bacteria counts than in silage that was ensiled for 30 days. In conclusion, high CP, moderate fibre and better *in vitro* fermentation characteristics in *P. purpureum* ensiled with treated *E. cyclocarpum* seeds at varying proportions and at different ensiling periods suggests better nutritive alternatives to sole *P. purpureum* grass silage, as well as an appropriate feeding strategy which can improve ruminants' performance especially during the dry season

Keywords: Enterolobium cyclocarpum seeds, fermentation, nutritive value, processing, proportion, Pennisetum purpureum.

EVALUATION DES EFFETS DE LA PROPORTION ET DE LA DUREE D'ENSILAGE SUR LA DIGESTIBILITE ET LES CARACTERISTIQUES DE FERMENTATION DES ELEMENTS NUTRITIFS DES ENSILAGES DE PENNISETUM PURPUREUM ET D'ENTEROLOBIUM CYCLOCARPUM EN UTILISANT LA TECHNIQUE DE PRODUCTION DE GAZ IN VITRO

Resume

La valeur nutritive de l'herbe de Pennisetum purpureum avec ensilage de graines d'Enterolobium cyclocarpum traitées à des proportions variables et à différentes périodes d'ensilage a été évaluée sur la base de leur composition chimique et de leur fermentation in vitro. L'étude consistait en une expérience factorielle 7 × 2 avec trois répétitions. Les facteurs étaient sept proportions différentes - (100: 0) comme contrôle, 85:15; 70:30) pour des graines de E. cyclocarpum bouillies, grillées et crues ensilées avec de l'herbe

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de P. purpureum à des proportions variables (85:15, 70:30 respectivement) et deux périodes d'ensilage (30 et 60 jours). Les résultats ont révélé que la teneur en protéines brutes (PB) de l'ensilage variait de 9,92 à 15,47%, les pourcentages 30% de graines bouillies + 70% d'herbe montrant les plus fortes teneurs en protéine brute. L'ensilage de 60 jours avait une teneur en PB significativement plus élevée (P <0,05) par rapport à celui de 30 jours. La gamme d'acides gras volatils dans le surnageant de l'ensilage après incubation in vitro pendant 48h était : acétate (0,74 à 1,90%) ; propionate (0,49 à 1,26%) et butyrate (0,07 à 0,19%). La digestibilité in vitro de l'ensilage variait entre 60,33 et 81,67% pour la matière sèche, 58,12 et 84,98% pour la fibre brute et la fibre détergente neutre entre 68,17 et 88,13%. L'ensilage avec 30% de graines crues + 70% d'herbe avait des nombres totaux de bactéries anaérobies significativement plus élevés (p> 0,05). L'ensilage des graines et de l'herbe à 60 jours a produit des nombres de bactéries anaérobies totaux plus élevés par rapport à l'ensilage de 30 jours. En conclusion, une PB élevée, une teneur modérée en fibres et de meilleures caractéristiques de fermentation in vitro chez P. purpureum ensilées avec des graines d'E. Cyclocarpum traitées à des proportions variables et à différentes périodes d'ensilage semblent indiquer de meilleures alternatives nutritives par rapport à l'ensilage d'herbe pure P. purpureum, ainsi qu'une stratégie d'alimentation appropriée qui peut améliorer la performance des ruminants en particulier pendant la saison sèche.

Mots-clés: graines d'Enterolobium cyclocarpum, fermentation, valeur nutritive, traitement, proportion, Pennisetum purpureum.

Introduction

During the dry season, ruminants' diets are limited by shortages in amount and quality of available forage, crop residues or by-products (Babayemi *et al.,* 2004). This results in reduced livestock productivity (Odenyo *et al.,* 1997). Moss (1993) recommended the use of concentrate feeds for ruminants but scarcity and/or high cost of such feeds have limited their use by farmers. Tropical forages are high in quantity during the wet season which outweigh the livestock requirements. Conservation of excess forages produced during rainy season and their use as supplements during the dry season will go a long way in alleviating the problems of forage scarcity and of low quality (Ojo *et al.,* 2014). Tropical grasses have been reported to grow and mature earlier than their counterpart with the same age in temperate region as a result of high temperature regime. This however, stimulate growth and aging of the plants which lead to fall in nutrients and digestibility (Ojo *et al.,* 2016). Silages made from such lignified tropical grasses alone are poor in nutrients because of the low protein content (Babayemi and Igbekoyi, 2008). In other to utilize these grasses as livestock feeds, increasing the nutrient density with a rich protein source as supplement will improve fermentation and the nutritive value of the silages. Forage seeds have been reported to be high in crude protein and as such are suitable as protein source which hold nutritional promise for optimum performance in ruminants (Babayemi and Bamikole, 2006). Seeds from browse trees are not being employed as feed ingredient in the tropics but are quite abundant and can be preserved throughout the year. Since the seeds are high in crude protein content, this will help the utilization of fermentable carbohydrate by lactic acid bacteria and simultaneously increase the protein content of the silage (Assefa and Ledin, 2001).

Browse seeds possess secondary metabolites which limits their utilization by ruminants, as such, *in vitro* gas production can be employed for the nutritional evaluation of such seeds (Babayemi, 2009). Gas production technique is a quick and less expensive means of determining the beneficial value of feeds for ruminants (Babayemi and Bamikole, 2006) and the information from the procedure help give a better evaluation of the nutritive potential of such feeds (Arigbede *et al.,* 2012). Prediction of methane (CH4), volatile fatty acids (VFA) and the individual molar VFA can be done via gas production (Fievez *et al.,* 2005). The objective of the present study was to determine the chemical composition and *in vitro* fermentation characteristics of *P. purpureum* grass ensiled with treated *E. cyclocarpum* seeds at varying

proportions and at different ensiling periods.

Materials and Methods

Location and climate of the study area

The experiment was carried out at the College of Animal Science and Livestock Production farm, Federal University of Agriculture, Abeokuta, Nigeria in November, 2014. The area lies within the Savannahh agro-ecological zone of south western Nigeria (Latitude 7o 13 49.46 N, longitude 3o 26 11.98 E, average annual rainfall of 1037mm).

Sourcing, collection and processing of test ingredients

Matured pods of *E. Cyclocarpum* were picked after falling off from the tree stands. The collected pods were sun-cured for three days and dehulled to obtain the seeds. The seeds were then divided into three portions. One portion was raw seeds which served as control, another was boiled at 100°C for 60 minutes to give boiled seeds and the third portion was toasted at 170°C for 15 minutes to give toasted seeds. The differently processed seeds were ground to pass through 2mm sieve. Pennisetum purpureum was harvested at 6 weeks old froman established plot in the study area. Defoliation of the grass was done at 20cm above ground level, wilted for four hours and chopped into 2cm long. The grass and the seeds were mixed at different proportions as follows:

1. 100% of *P. purpureum*: 0% of *E. cyclocarpum*,
2. 85% of *P. purpureum* : 15% of raw *E. cyclocarpum* seeds
3. 70% of *P. purpureum* : 30%of raw *E. cyclocarpum* seeds
4. 85% of *P. purpureum* : 15% of boiled *E. cyclocarpum* seeds
5. 70% of *P. purpureum* : 30%of boiled *E. cyclocarpum* seeds
6. 85% of *P. purpureum* : 15% of toasted *E. cyclocarpum* seeds
7. 70% of *P. purpureum* : 30 % of toasted *E. cyclocarpum* seeds

These were ensiled for 30 and 60 days in laboratory bottles (960 ml) at an ambient temperature of 26°C. Each treatment had three replicates.

Experimental design

The study was a 7×2 factorial experiment. The factors were seven different proportions (100:0 as control, 85:15; 70:30) for boiled, toasted and raw *E. cyclocarpum* seeds ensiled with *P. purpureum* grass at varying proportions (85:15; 70:30 respectively) and two ensiling periods (30 and 60 days).

Chemical analyses

At the end of 30 and 60 days ensiling periods, the silos were opened and sub samples of 300g were collected and oven dried at 65oC until constant weight was obtained and ground to pass through 1mm sieve using laboratory Thomas-Wiley mill and analysed for proximate composition (A.O.A.C. 1995, reference ID numbers DM 930.15, CP 984.13, Ash, 942.05 and EE 963.15). The neutral detergent fibre was determined asdescribed by Van Soest *et al.,* (1991). Tannin content was determined using the Vanillin-HCl method as described by Price and Betler (1977).

In vitro fermentation analysis

The *in vitro* gas production was determined following the procedure of Menke and Steingass (1988). Two hundred (200) mg of the milled samples were measured into fibre bags, tied and put into 100 ml glass syringes fitted with plungers. Each sample was replicated ten times. Macro- and micro-elements, reduction and resazurin dye solutions were mixed together with distilled water and the pH adjusted to 6.9 with buffer solution. Rumen fluid was collected from three bulls according to the method described by Babayemi and Bamikole (2006). The animals were fed *P. purpureum* hay and concentrates. The rumen fluid was taken to the laboratory, sieved with four layers cheese cloth and added to the anaerobic buffer medium. Thirty (30) ml of the mixture was then added to each syringe. The syringes were placed in a refrigerated incubator and the temperature regulated to 40°C for 48 h. After the incubation period, fermentation residues

in fibre bags of each syringes were thoroughly washed with distilled water and dried at 105 0C for 24 h. The oven-dried residues were then used for the evaluation of dry matter, crude protein and neutral detergent fibre according to AOAC (2010) to determine *in vitro* nutrients digestibility.

Thirty (30) ml of the supernatants of *in vitro* rumen liquor after the incubation period were decanted into plastic bottles and each divided into two sets. The first set was used for the determination of ammonia and volatile fatty acids analyses using AOAC (2010) and Samuel et al. (1997) procedures respectively. The second sub-portion was fixed with 10% formalin solution in a sterilized 0.9% saline solution for the enumeration of total bacteria, protozoa and fungi counts according to the method of Baker and Breech (1986).

Statistical Analysis

All data obtained were subjected to two-way analysis of variance (ANOVA). Means were separated using Duncan's Multiple Range Test (SAS, 1999) package.

Results

Chemical composition

All the parameters measured except ash were significantly different (Table 1). The DM content of the silage ranged (P<0.05) from 88.17 to 95.17 %. The silages had relatively high CP concentrations with the highest recorded for silage made from 30% boiled seeds+70% grass (15.47 %). NDF was higher for silage produced from 30% toasted seed + 70% grass, and the least value was recorded for those made from 15% toasted seed + 85% grass. Silage produced from 100% *P. purpureum* without *E. cyclocarpum* seed inclusion was observed for higher NFC content than other treatments. The chemical composition of the silage was not affected by ensiling periods except for CP which was higher for 60 days (13.16%) than 30 days ensiling periods. In contrast, tannin content was higher for the silage ensiled for 30 days than its 60 days counterpart.

Ruminal fluid parameters

Ensiled Pennisetum with treated Enterolobium seeds silage at different proportions had significant (P<0.05) effect on ruminal fluid parameters (Table 2). There was a significant difference in the ammonia nitrogen of the silage, with Pennisetum ensiled with *E. cyclocarpum* seeds silage recording higher values than those made from sole Pennisetum. Acetic, butyric, propionic and volatile fatty acids were higher for 30% toasted seed + 70% grass silage and the lowest content of these acids were recorded for the silage produced from 0% raw seed + 100% grass.

Storage of the silage for 30 days resulted in significantly higher (P<0.05) values for acetic, butyric and propionic acids above 60 days ensiling period. Meanwhile, 60 days ensilage period had higher VFA and NH3-N values than in 30 days period. In addition, the interaction effects of proportion and ensiling period was significant on all the rumen fluid parameters.

In vitro nutrient digestibility

There were significant differences (P<0.05) in the *in vitro* nutrients digestibility of ensiled Pennisetum with treated Enterolobium seeds silage at different proportions (Table 3). Higher DMD was observed for silage made from sole grass with no legume seed inclusion while the least values was recorded for silage produced from 15% raw seed + 85% grass (603.30 g/kg) and 15% boiled seed + 85% grass (603.30 g/kg) respectively. The digestibility of crude protein was (P<0.05) higher for 30% toasted seed + 70% grass silage than other treatments. No difference was observed in the Neutral detergent fibre digestibility (NDFD) of 15% raw seed +85% grass and 30% boiled seed + 70% grass silages, but were significantly higher than other treatments. However, silage produced from 15% boiled seed + 85% grass and 30% toasted seed + 70% grass recorded statistically similar values. Ensiling period did not (P>0.05) affect the nutrient digestibility of ensiled Pennisetum with treated Enterolobium seeds silage. At the interaction level, CPD and NDFD of the silage differs while DMD was not

Table 1: Chemical composition of Pennisetum purpureum grass with treated Enterolobium cyclocarpum seeds silage at varying proportions and different ensiling periods

Factors	DM	CP	Ash	EE	NDF	NFC	Tannin
			(%)				(mg/kg)
Proportions (%)							
0% raw seed + 100% grass	91.83^{bc}	9.92^{e}	63.39	5.67^{d}	38.22^{b}	39.85^{a}	4.67^{g}
15% raw seed + 85% grass	88.17^{d}	12.28^{d}	59.18	8.08^{bc}	35.77^{b}	37.95^{a}	6.58^{e}
30% raw seed + 70% grass	89.50^{cd}	14.26^{b}	56.19	10.17^{a}	36.22^{b}	33.72^{b}	5.25^{f}
15% boiled seed + 85% grass	91.83^{bc}	13.38^{c}	58.11	8.89^{ab}	35.55^{b}	36.37^{ab}	8.83^{d}
30% boiled seed + 70% grass	92.83^{ab}	15.47^{a}	54.07	7.83^{bc}	31.88^{c}	39.40^{a}	9.50^{c}
15% toasted seed + 85% grass	95.17^{a}	12.35^{d}	58.00	7.21^{c}	35.33^{b}	39.31^{a}	9.75^{b}
30% toasted seed + 70% grass	93.00^{ab}	14.22^{b}	55.00	9.29^{ab}	42.44^{a}	28.54^{c}	11.67^{a}
SEM	0.826	0.131	0.377	0.524	1.102	1.153	0.023
Ensiling periods (days)							
30	93.14	12.94b	59.73	7.33	39.19	34.56	11.93^{a}
60	92.57	13.16a	57.67	8.06	39.81	33.20	8.43^{b}
SEM	0.607	0. 374	0.227	0.411	1.305	1.465	0.015
p-value							
Proportions	0.000	0.000	0.670	0.000	0.000	0.000	0.000
Ensiling period	0.000	0.032	0.520	0.003	0.000	0.000	0.000
Proportion x Ensiling period	0.026	0.999	1.000	0.984	0.000	0.000	0.000

$^{a-g}$: Means in same column with different superscripts are significantly ($p<0.05$) different.
SEM = standard error of mean,DM =dry matter, CP = crude protein, EE = ether extracts, NDF = Neutral detergent fibre, NFC = Non-fibre carbohydrate.

Table 2: *In vitro* volatile fatty acid, pH and ammonia concentrations of Pennisetum purpureum grass with treated Enterolobium cyclocarpum seeds silage at varying proportions and at different ensiling periods

Factors	Acetic acid (%)	Butyric acid (%)	Propionic acid (%)	Volatile fatty acid (%)	Ammonia N (%)	Temp (^{0}C)	pH
Proportions (%)							
0% raw seed + 100% grass	1.35^{c}	0.14^{c}	0.90^{c}	103.00^{e}	11.25^{e}	22.70^{a}	6.51^{a}
15% raw seed + 85% grass	1.37^{b}	0.14^{b}	0.92^{b}	106.00^{d}	17.09^{a}	22.45^{b}	6.06^{b}
30% raw seed + 70% grass	1.38^{b}	0.14^{b}	0.92^{b}	107.00^{d}	13.09^{d}	22.05^{d}	5.70^{c}
15% boiled seed + 85% grass	0.93^{e}	0.09^{e}	0.62^{e}	140.00^{b}	14.54^{c}	22.10^{d}	5.63^{c}
30% boiled seed + 70% grass	1.22^{d}	0.12^{d}	0.81^{d}	183.00^{a}	16.16^{b}	22.45^{b}	5.66^{c}
15% toasted seed + 85% grass	0.74^{f}	0.07^{f}	0.49^{f}	111.00^{c}	13.01^{d}	22.40^{b}	6.11^{b}
30% toasted seed + 70% grass	1.90^{a}	0.19^{a}	1.26^{a}	184.00^{a}	13.18^{d}	22.25^{c}	5.71^{c}

Factors	Acetic acid (%)	Butyric acid (%)	Propionic acid (%)	Volatile fatty acid (%)	Ammonia N (%)	Temp (^{0}C)	pH
*Rumen fluid	0.50	0.05	0.38	76.00	8.28	28.8	5.54
SEM	0.10	0.01	0.07	0.15	0.64	4.80	0.07
Ensiling periods (days)							
30	1.59a	0.16a	1.06a	138.00b	11.18b	22.43a	5.87
60	0.95b	0.09b	0.63b	143.00a	17.20a	22.26b	5.95
SEM	0.1	0.01	0.07	1.50	0.86	0.06	0.10
P- value							
Proportion	0.0015	0.0015	0.0015	0.0015	0.0012	.0001	0.3086
Ensiling period	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Proportion x Ensiling period	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

*[a-f]:Means in same column with different superscripts are significantly (p<0.05) different.SEM= Standard error of mean. *R= was not statistically analysed*

Table 3: Nutrients digestibility in *in vitro* incubations of Pennisetum purpureum grass with treated Enterolobium cyclocarpum seeds silage at varying proportions and at different ensiling periods

Factors	Dry Matter Digestibility	Crude Protein Digestibility	Neutral Detergent Fibre Digestibility
		g kg^{-1}	
Proportions (%)			
0% raw seed + 100% grass	816.70^{a}	699.80^{bcd}	681.70^{c}
15% raw seed + 85% grass	603.30^{b}	724.80^{bc}	881.30^{a}
30% raw seed + 70% grass	610.00^{b}	687.80^{cd}	800.00^{b}
15% boiled seed + 85% grass	603.30^{b}	687.40^{cd}	853.30^{ab}
30% boiled seed + 70% grass	643.30^{b}	581.20^{d}	865.00^{a}
15% toasted seed + 85% grass	646.70^{b}	765.50^{b}	808.30^{b}
30% toasted seed + 70% grass	683.30^{b}	849.80^{a}	850.00^{ab}
SEM	8.00	3.74	5.00
Ensiling periods (days)			
30	660.00	656.10	857.60
60	656.20	673.20	835.70
SEM	1.52	2.92	0.79
P –value			
Proportion	0.0001	<.0001	0.0181
Ensiling period	0.9018	0.7745	0.1657
Proportion x Ensiling period	0.0012	<.0001	<.0001

[a-d]:Means in same column with different superscripts are significantly (p<0.05) different.SEM= Standard error of mean.

Table 4: *In vitro* microbial counts of Pennisetum purpureum grass with treated Enterolobium cyclocarpum seeds silage at varying proportions and at different ensiling periods

Factors	Total anaerobic bacteria count	Total fungi count	Total protozoa count
	0^6cfu/ml		10^3 cell/ml
Proportion (%)			
0% raw seed + 100% grass	0.80^{c}	0.10^{a}	1.15^{c}
15% raw seed + 85% grass	0.70^{cd}	0.05^{b}	2.15^{b}
30% raw seed + 70% grass	2.7 0^{a}	0.05^{b}	0.05^{d}
15% boiled seed + 85% grass	2.10^{b}	0.00^{c}	3.65^{a}
30% boiled seed + 70% grass	0.35^{d}	0.10^{a}	0.60^{cd}
15% toasted seed + 85% grass	0.60^{c}	0.10^{a}	3.80^{a}
30% toasted seed + 70% grass	0.30^{d}	0.00^{c}	2.15^{b}
*RUMENLIQUOR	0.30	0.01	2.1
SEM	0.29	0.02	0.39
Ensiling periods (days)			
30	0.59^{b}	0.04^{b}	2.01^{a}
60	1.57^{a}	0.07^{a}	1.86^{a}
SEM	0.23	0.01	0.39
P –value			
Proportion	<.0001	<.0001	<.0001
Ensiling period	<.0001	0.0005	0.4923
Proportion x Ensiling period	<.0001	<.0001	<.0001

*$^{a-d}$:Means in same column with different superscripts are significantly ($p<0.05$) different.SEM= Standard error of mean.*R= was not statistically analysed, colony forming unit (cfu)*

influenced by the interactions of the factors under study.

In vitro microbial counts

The total anaerobic bacteria count differs across the treatments with the highest count observed for 30% raw seed + 70% grass silage (Table 4). There was no difference in the total bacterial count for 30% boiled seed + 70% grass and 30% toasted seed + 70% grass silages, but were statistically lower than other treatments. For total fungi count, similar ($P<0.05$) higher values was recorded for 0% raw seed + 100% grass, 30% boiled seed + 70% grass and 15% toasted seed + 85% grass silages respectively. In contrast, 15% boiled seed + 85% grass and 30% toasted seed + 70 grass silages were observed for significantly lower total fungi count. The total protozoa count as affected by varying levels of *E. Cyclocarpum* seeds and *P. purpureum* silage incubated for 48 h ranged from 0.05 to 3.80 x103cell/ml.

Higher total bacterial count and total fungi count was recorded for the silage ensiled for 60 days compared to those ensiled for 30 days, while total protozoan count took the inverse pathway. The interaction effect of the factors imposed in this study was significant on the *in vitro* microbial count of Pennisetum purpureum grass with treated Enterolobium cyclocarpum seeds silage.

Discussion

The CP values obtained in this study showed an increasing trend as the inclusion of processed *E. cyclocarpum* seeds increased in the silage mixture. In a similar trend, Babayemi and

Igbekoyi (2008) reported that CP increased in silage with the increase in the inclusion of Albizia saman pods in ensiled guinea grass mixture. The CP contents in this study were generally above the critical limit at which forage intake by ruminants and rumen microbial activity could be negatively affected (Van Soest, 1994).The increase in the CP content of the silages recorded in this study as influenced by the ensiling periods disagreed with the report by Jolaosho et al. (2013) when Guinea grass, cassava peel and brewery waste were ensiled at different proportions. The difference could be as a result of inclusion of *E. cyclocarpum* seeds in the present study which have been reported to be high in crude protein content (Babayemi *et al.,* 2004).

The neutral detergent fibre (NDF) contents of silage in this study was lower than the range of 600-650 gkg-1 suggested as the critical limit above which efficiency of utilization of tropical forages by ruminants would be impaired (Muia, 2000). The range of values obtained for NDF in all silage proportions in this study is in consonance with the values recorded by Arigbede et al. (2011) for multi-purpose plants.

The values obtained for ammonia nitrogen in Pennisetum ensiled with *E. cyclocarpum* seeds silage was higher than that recorded for sole Pennisetum silage. This increase could be as a result of increased level of protein fermentation due to additional protein supplied by the seeds. The ammonia nitrogen (NH3-N) in this study was higher than 12.65-15.42 mg/dl reported by Akinbode et al. (2014) for tropical grasses and crop residues while the authors reported lower acetic (66.35-70.85 mol/100mol) and propionate acids (20.75-23.4 mol/100mol) compared with those recorded in the present study. Leng et al. (1977) reported optimum rumen ammonia concentration of 5-20 mg NH3/100ml which is comparable with the result obtained in the present study.

The high volatile fatty acid (VFA) production was, however, in agreement with earlier reports for VFA contents in tropical forages by Odenyo et al.(2003) and Mbugua et al.(2008). According to Mbugua et al.(2008), tannins and alkaloids in forages can adversely affect their fermentation characteristics, thereby reducing their nutritive values. The levels of VFA reported in the current study showed that the level of tannins in the seeds was within the range that does not reduce the bioavailability and utilization of nutrients present in them. Since the pH values were regulated with buffer medium, the near neutral pH values recorded in this study was expected. The pH range obtained across the treatments in this study were within the normal range reported as optimal for microbial digestion of fibre (Firkins, 1996) and also for digestion of protein in the rumen (Wanapat, 1999).

Sixty days ensilage had higher VFA and NH3-N values than for 30 days period. The variation in NH3-N concentration might be due to the higher crude protein content at 60 days ensiling period which might have influenced nitrogen uptake by the rumen microbes (Okoruwa, 2015).

The nutrients digestibility values of the silage in this study were higher than the findings of Nha et al. (2008) who reported values of 508 -520 g/kg, 615- 632g/kg and 554- 565g/kg, for DMD, CPD and NDFD respectively for cottonseed meal and foliage of Sesbania grandiflora and urea that were fed to cattle and buffaloes. Sole (100%) *P. purpureum* silage with highest dry matter digestibility (81.67%) above others, could be because the rumen microbes which aid digestion in ruminants were accustomed to the grass, as grasses form the basal part of their diets. Moderate levels of NDF may be responsible for generally high IVDMD in the silage (Njidda, 2014). The lowest IVDMD value obtained from 15% raw seeds + 85% grass, may be due to the presence of anti-nutritional factor which could limit the activities of rumen microbes that aid digestion (Babayemi, 2006). The mean DMD in this study compared favourably with mean value of 650.70 g/kg reported by Hanlin et al. (2011) for several tropical legumes in China. When IVDMD falls below 55% there is physical limitation on the rate of eating and the rate of digestion in ruminants. This invariably make the passage of feed through the gastrointestinal tract to

be restricted while live weight loss becomes inevitable (SCA, 1990). Based on this, the generally high IVDMD recorded in this study demonstrated the high nutritive potential of the ensiled forages if used in livestock feeding.

The range of CPD of the silage in this study was higher than 578 g/kg reported for diets of Panicum maximum that was fed in combination with Gmelina arborea leaves in the ratio of 60:40 in WAD bucks (Aderinboye *et al.,* 2012). The highest CPD in 30% toasted seeds + 70% grass could be as a result of higher content of the seeds in the silage, which might have increased its quality. Inclusion of differently treated *E. Cyclocarpum* seeds to *P. purpureum* silage in this study increased the CPD which was in the range of 340g/kg in *Phaseolus lunatus* to 883g/kg in Lenis esculenta reported by Jaffe (1950).

Oba and Allen (1999) reported that *in vitro* NDFD can vary considerably among and within laboratories because it is a biological assay that is relatively complex and because the ruminal fluid used in these assays could vary within and among donor animals. Even with this variability, *in vitro* NDFD in this study were typically high. It was however, observed that dry matter digestibility increased with increase in seed inclusion in the silage. Nevertheless, degradation in the rumen have been reported to increase the absorption of essential amino acids from the small intestine (Foiklang *et al.,* 2011).

Microbial yield in the rumen is very important because it is an index or a function of the amount of microbial protein made available in the first stomach compartment of ruminants daily (Okoruwa, 2015). Low values were obtained for fungi count in this study, unlike the report of Okoruwa (2015) where a high amount of fungi count (34.35-40.26 mm2) was observed when growing rams were fed avocado seeds with orange peels meal as replacement for guinea grass. The variations in the microbial counts might be due, in part, to differences in feed materials used in the former and later studies.

The values obtained from this study showed that increase in the proportion of *Enterolobium* seeds in the silage mixture resulted in lower *protozoan* contents obtained in the digested rumen supernatant of this study. The low protozoa count with increased Enterolobium seeds levels could be as a result of the presence of tannin in the seeds which have been reported to play important role in decreasing protozoa populations. In general, tannin could lower methanogenesis through reductions of number of protozoa and methanogens (Patra and Saxena, 2009). Makkar *et al.,* (1995) reported that condensed tannin altered rumen ecology and increased microbial protein synthesis. Consequently, reduction of protozoa and methanogens population could decrease greenhouse gases. Results for protozoa count in this study were lower than 21-23 x 105 ml-1 reported by Wina *et al.,* (2006) when Sapindusrarak saponins was fed to sheep. The difference in protozoa count might be as a result of differences in feed materials used as well as the level of anti-nutritional factors present.

The higher levels of tannin (Salem *et al.,* 2006) in silages with 30% toasted seeds + 70% grass, 15% toasted seeds + 85% grass, 30% boiled seeds + 70% grass and 15% boiled seeds + 85% grass could have been responsible for the reduction in total bacteria and fungi counts compared to the other treatments. Tannin exerts adverse effects on ruminal microbes (McSweeney *et al.,* 2005). This agreed with the report by Salawu et al. (1999) that high level of tannins could reduce the activities of bacteria. This can be due to reduction in the attachment of micro-organisms to feed particles (McAllister *et al.,* 1994), inhibition of microbial growth and enzyme activity (McSweeney *et al.,* 2005).

Anaerobic fungi are reported to be the first to reduce the tensile strength of feed particles and increase the surface area of particles for breakdown in rumination, thus they are important initiators of fermentative breakdown of insoluble plant cell wall materials (Okoruwa *et al.,* 2013). The low fungi counts obtained in the digested rumen supernatant of this study could be due to low fibre content of the silage. Bauchop (1979) reported that fungi were more prevalent in ruminants fed high

fibre diets than in those fed less fibrous ones.

The proliferation of bacteria is associated with extensive fermentation in the rumen. This was observed in silage that was ensiled for 60 days where increased fermentative activities, could have been responsible for the high digestion of more protein and fibre. This could have been possible by the microbes attaching to plant particles to provide more NH3-N concentration and total volatile fatty acids which enhance microbial activities.

Conclusion

Treated *E. cyclocarpum* seeds ensiled with *P. purpureum* grass at different proportions are potential source of readily available feeds that would fill the gap of forage shortage for ruminants during the dry season. Based on the results obtained in this study, it can be concluded that enriching *P. purpureum* grass with treated *E. cyclocarpum* seeds will offer a balance of essential nutrients required for ruminants. Therefore, inclusion of 30% boiled *E. Cyclocarpum* seeds with 70% *P. purpureum* grass ensiled for 60 days is recommended as an appropriate feeding strategy which can improve ruminants' performance especially during the dry season without any negative effects on rumen microbial population and volatile fatty acids.

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SHORT COMMUNICATION

EFFECT OF PHYSIOLOGICAL STATUS ON HAEMATOLOGICAL PARAMETERS OF WEST AFRICAN DWARF GOATS

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Introduction

Pregnancy and lactation are physiological stages thought to induce metabolic and hormonal stress (Celi *et al.,* 2008). In most practical production systems, farmers battle with neonatal death, post-parturition complications which give rise to a debilitation of does health and a distorted breeding programme (Obidike *et al.,* 2009). Joshi *et al.,* (2002) reported the quality and quantity of blood are being regarded as an important indicator in determining the health status of animals. Blood acts as a pathological indicator of the whole body and hence haematological parameters are important in diagnosing the functional status of an exposed animal to suspected toxicant. Therefore the haematological analysis done at the onset of the experiment serves as a reference point and a basis to ascertain the health status of the animals for the experiment.

Estimation of *heamatological* parameters are helpful complementary diagnostic tools (Kaneko *et al.,* 1997) and form the basis of metabolic profile tests which help to prevent the occurrence of several metabolic disease (Celi *et al.,* 2008). It is essential to gain insight into physiological changes in the female animal if a complete understanding and possible manipulation of reproductive physiology is desired.

Materials and Methods

The study was conducted in Western Nigeria (7 10N, 3 2'N) at the Teaching and Resaerch Farm, Federal University of Agriculture, Abeokuta, Nigeria located at 76m above sea level. Twelve lactating West African dwarf goats homogenuous for age (2-3years), liveweight (LW21.06± 0.94kg). The female goat becomes mature when it reaches 10-12 months and is chracterised by high percentage of twinning. They were fed panicum maximum ad libitum with supplementation of cassava peel, palm kernel cake and wheat offal. Ectoparasites and endoparasites were routinely controlled, together with prescribed vaccinations. The goats were oestrous sychronised naturally and mated so that kidding occurred within a month. Pregnancy was diagnosed and confirm by abdominal palpation and the use of a portable ultrasound machine.

Blood samples were collected from the jugular vein using a sterile needle and a syringe. The blood samples were collected into a bijour bottles with EDTA for haematological analysis. The collected sample were immediately placed in a coolong jar and promptly transported to the of Veterinary medicine laboratory. The packed cell volume (PCV), red blood cell count, white blood cell and differential count were determined using standard procedures (Cole, 1986).

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Statistical analysis: The data were subjected to One-way analysis of variance (ANOVA), the means were separated using Duncan's multiple range.

Result and Discussion

The range of values obtained for Packed cell volume (PCV) in this study compared favourably with the values 28-33% reported by Igado *et al.,* (2011) during the investigation into the haematological and liver enzymes changes of WAD goats fed cassava peel at different stages of gestation (although the different stages of gestation were not considered in this study). However the values fell within the normal range of 22-38% reported by Oni *et al.,* (2010) and Merck (2011). There were slight increases in the PCV values of the treatment groups during late gestation when compared to the values obtained before gestation. This indicates that contrary to the common situation in humans, pregnancy does not exert any negative influence on PCV values in pregnant ewes (Durotoye and Oyewale, 2000). The range of values of PCV was higher at gestation when compared to early lactation. This result is contrary to the results of (Habibu *et al.,* 2014) who reported a higher PCV in lactating does compared to pregnant does. This decreased PCV during early lactation might be ascribed to the increasing water mobilization to mammary gland through the vascular system (El-Sherif and Assad, 2001).

In this study the range of red blood cell values observed were in accordance with the recommended values for normal blood parameters in sheep 9-15 (x109/l) (Merck, 2015). There was a progressive decrease in the value of RBC counts observed when the range of RBC values during late gestation and at early lactation were compared and this is consistent with the findings of Makinde *et al.,* (1983) who reported the same in goats after the withdrawal of 30% of the calculated blood volume via the jugular vein to stimulate hemorrhage. These observations are due to the movement of intestinal fluid into the vascular system to replace fluid volume lost through hemorrhage (Oyewale *et al.,* 1997). In this study, the blood loss was due to parturition.

The range of values recorded for *haemoglobin* (Hb) was slightly higher than the 7.53-10.33g/dl reported by Igado *et al.,* (2011). However the Hb concentration fell within the normal range of values of 8-15g/dl indicated by Oni *et al.,* (2010). The relatively higher Hb concentration obtained in this study suggests that the dietary treatments generally seemed to be capable of supporting high oxygen carrying capacity blood in the goats. Furthermore the range of values of Hb was higher during early lactation when compared to the values obtained during late gestation and before gestation. This is in contrast to the work of Durotoye and Oyewale (2000) who reported the highest mean value of Hb (12.07±1.40 g/dl) during gestation of ewes. The low Hb observed during late gestation in this study may have resulted from haemodilution which is a normal physiological response during late gestation directed at decreasing blood viscosity so as to enhance blood supply to small blood vessels (Guyton and Hall, 1996). This is aimed at satisfying the demand of the new vascular bed since some amount of blood must occupy spaces in the uterus and maternal placenta and also to compensate for the expected blood loss during delivery (Ramsay 2010).

The range of WBC values observed in this study were contrary to the range of 6.8-20.1(×109/l) reported by Daramola *et al.,* (2005) but however fell within the normal reference range of values reported by (RAR, 2009; Etim *et al.,* 2014). The range of white blood cells obtained in this study during late gestation was lower when compared to the range before gestation but however increased during early lactation. This result is in accordance with a study on Tsigai ewes by Antunovic *et al.,* (2011a), suggesting that the low number of total WBCs count during pregnancy and the increase at parturition and early lactation is probably a response to uterine involution.

The range of values for neutrophils observed in this study is in contrast to the values of (17-52%) reported by Daramola et al. (2005) but however fell within the recommended

Table 1: Mean haematological (±SE) indices of West African Dwarf does at early, third trimester of pregnancy and early lactation

	Control	Late pregnancy	Early lactation	Mean±SE
Weight (kg)	16.92±1.03[b]	22.50± 1.51[a]	23.75± 1.61[ab]	21.06± 0.94
Packed cell volume (%)	26.00 ± 1.07[b]	29.83 ± 1.19[a]	27.25 ±1.09[ab]	27.69 ± 0.68
Red blood cell (%)	11.58 ± 0.82[a]	9.99 ± 0.42[ab]	9.25 ± 0.36[c]	10.27 ± 0.36
Haemoglobin (%)	8.83 ± 0.41[b]	10.88± 0.88[ab]	13.12± 1.20[a]	10.94± 0.58
White blood cell(%)	16.10± 0.72[a]	10.23± 0.71[b]	14.53± 1.09[a]	13.62± 3.83
Neutrophils (%)	43.75 ± 3.56[ab]	48.75 ± 3.40[a]	36.92 ±3.69[b]	43.14 ± 2.15
Lymphocytes (%)	52.92± 3.44[ab]	47.67± 3.63[b]	60.00± 4.01[a]	53.53± 2.25
Monocytes (%)	1.58± 0.48	2.08± 0.40	1.08± 0.47	1.58± 0.26
Eosinophils (%)	1.42± 0.42	1.17± 0.32	1.50± 0.54	1.36± 0.25
Basophils (%)	0.17± 0.11	0.33± 0.14	0.58± 0.36	0.36± 0.13

[a,b,c] means with a different superscript in a different column are significantly different (P<0.05)

range of values of 0-50% (Merck Veterinary manual 2015). These values are suggestive of a well-developed immune system in the WAD goats with such immune cells to proffer good health (Daramola *et al.,* 2005).

The values obtained in this study for lymphocytes fell within the broad range of 47-82% reported by Daramola *et al.,* (2005). The range of values for lymphocyte were higher during early lactation compared to the values obtained during late gestation and this is in consonance with the findings of Obidike *et al.,* (2009) who reported a higher lym¬phocyte count postpartum. This increase in lymphocyte count could be due to physiological stress induced by lactation.

The *monocytes* and *basophiles* counts were minimal in blood of does during the three different physiological stages and the values obtained fell within the broad range of 0-6 and 0-3% reported by (Merck, 2015) for monocyte and basophil respectively. However, there are several reports suggesting no effect of reproductive status and sex on total leucocyte exist (Daramola *et al.,* 2005; Iriadam 2007). In general, the varia¬tions observed between haematological parameters in this study and those reported by other authors, could be due to differences in breed, age, parity, species, sex, blood collection procedure, animal housing and subclinical illness. However Togun *et al.,* (2007) reported that when the haematological values fall within the normal range reported for the animal, it is an indication that diets did not have any adverse effect on haematological parameters during the experimental period.

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AFRICAN UNION - INTERAFRICAN BUREAU FOR ANIMAL RESOURCES (AU-IBAR)

Bulletin of Animal Health and Production in Africa
Guide for Preparation of Papers
Notes to Authors

The Editor in Chief
March 2018

Aims and scope

The Bulletin of Animal Health and Production in Africa (BAHPA) of the African Union Interafrican Bureau for Animal Resources (AU-IBAR) is a scientific journal which publishes articles on research relevant to animal health and production including wildlife and fisheries contributing to the human wellbeing, food security, poverty alleviation and sustainable development in Africa. The bulletin disseminates technical recommendations on animal health and production to stakeholders, including policy makers, researchers and scientists in member states. The Bulletin is the African voice on animal resources issues specific to Africa.

The Bulletin of Animal Health and Production publishes articles on original research on all aspects of animal health and production, biotechnology and socio-economic disciplines that may lead to the improvement animal resources. Readers can expect a range of papers covering well-structured field studies, manipulative experiments, analytical and modeling studies of the animal resources industry in Africa and to better utilization of animal resources.

The BAHPA encourages submission of papers on all major themes of animal health and production, wildlife management and conservation, including:

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- Usefulness. Manuscripts submitted must help researchers, trainers, educators and policy makers in all regions of Africa improve their effectiveness.
- Rigorous methodology. Manuscripts submitted must be based on valid and reliable information, documentation or sound concepts, empirically, logically and theoretically supported.
- Well written to ensure clear and effective presentation of the work and key findings. The BAHPA editorial staff does not copyedit the text of accepted manuscripts, it is therefore important for the work, as presented, to be intelligible. Perfect, stylish language is not essential but it must be clear and unambiguous. If the language of a paper is not clear, Academic Editors should recommend that authors seek independent editorial help before submission of a revision. Poor presentation and language is a justifiable reason for rejection.
- Experiments, statistics, and other analyses performed are described in sufficient detail. The research must have been performed to a technical standard to allow robust conclusions to be drawn from the data. Methods and reagents must also be described in sufficient detail so that another researcher is able to reproduce the experiments described.
- Conclusions are presented in an appropriate fashion and are supported by the data. The results must be interpreted appropriately, such that all conclusions are justified. However, authors may discuss possible explanations for their results as long as these are clearly identified as speculations or hypotheses, rather than as firm conclusions. Inappropriate interpretation of results is a justifiable reason for rejection.
- The research meets all applicable standards for the ethics of experimentation and research integrity. Research to be published must have been conducted to the highest ethical standards. A brief description of the most common of these is described in our Editorial and Publishing Policies.
- Because the guidelines are updated as appropriate, authors should check them again before they submit their articles. Manuscripts submitted for publication will be considered for acceptance on the understanding that they present original work which has not been published or submitted for publication elsewhere and that they are subject to peer review.

Types of contribution

Full papers providing accounts of original work: Research containing significant new findings. The material presented should be original and not have been published elsewhere, except in a preliminary form. Papers will be reviewed by three referees familiar with the subject matter of the paper.

Short Communications: are intended to provide quick publication of highly relevant and interesting information. Manuscripts will be peer reviewed by two reviewers and the Editor.

Review Articles: should cover subjects falling within the scope of the bulletin, which are of active current interest. Papers need not contain original work or ideas. They will be reviewed for completeness, accuracy, style and suitability of content by referees familiar with the subject and the Editor-in-Chief..

Editorial: articles are short articles describing news about the bulletin or the opinion of the editor-in-chief, the publisher or a guest editor of a thematic series.

Letters to the Editor: the bulletin welcomes letters to the editor. The purpose of Letters to the Editor is to provide a forum for positive and constructive views on articles and matters published in the bulletin. Letters to the Editor must not exceed 300 words. Letters to the editors include technical reports from countries or projects.

Key notes and special calls: The editor will, from time, invite selected key figures in the field of animal health and production for key notes on specific topics. Book Reviews: are accepted and should provide an overview of the work's contents and a critique of the work's value. Book reviews should be limited to 1000 words.

Conference Proceedings: Special Issues of the bulletin may be dedicated to publication of proceedings of key meetings/ conferences

Obituary articles to honor prominent African scientists that have made significant contribution to animal resources research and development

News and announcements: BAHPA is pleased to publish information on animal health and production activities/meetings. Please send the following information to the Editor: Date of the event, title, organization offering the event, location and contact information.

Submission Guidelines
Full papers of original research

All manuscripts submitted to BAHPA should include the following features:

1. On cover page of the manuscript, the following should be clearly written/inserted: the corresponding author, name of the institution, , title of the manuscript, names of the authors, the addresses of the authors and the e-mail address of the corresponding author. The corresponding author should ensure that all the other authors consent to their names being included. The consent should be sent directly by co-authors to the editor via email.
2. Each original article should be divided into Abstract and Keywords, Introduction, Materials and Methods, Results, Discussion, conclusion, Acknowledgments and References. A textbox containing a public brief on the study for the benefit of policy makers should also be provided. This textbox will not be included in the published article but will be compiled and published in a separate edition at the end of the year.
3. Title, which should be concise, preferably not more than 15 words long, followed by the author(s) name(s) and institution(s) to which work should be attributed and address for correspondence, if different.
4. The Abstract should not be longer than 300 words giving a synopsis of the work and should contain the objectives, briefs description of materials and methods, highlights of significant results, conclusions and recommendations. Up to six keywords should be provided..
5. The Introduction should contain the problem statement, the hypothesis and the objective of the work and cite recent important work undertaken by others.
6. Materials and Methods should describe materials, methods, apparatus, experimental procedure and statistical methods (experimental design, data collection and data analysis) in sufficient detail to allow other authors to reproduce the results. This part may have subheadings. The experimental methods and treatments applied shall conform to the most recent guidelines on the animal's treatment and care. For manuscripts that report complex statistics, the Editor recommends statistical consultation (or at least expertise); a biostatistician may review such manuscripts during the review process. Cite only textbooks and published article references to support your choices of tests. Indicate any statistics software used.
7. Results should be presented clearly and concisely, in a non-repetitive way. Subheadings may be accepted.
8. Discussion of significance should be focused on in the interpretation of results. Subheadings are not accepted in this section.
9. Acknowledgements. Where necessary acknowledgements of grants and technical assistance should be included under this heading. . Please also include any potential conflict of interests if appropriate. Suppliers of materials should be named and their location (town, state/county, country) included.
10. State the conclusions, and any implications that may be drawn from the study.

Short Communications: Manuscripts should contain original data and be limited to 1500 words. The number of tables and figures are limited to two. A limited number of references should be included. Headings are not allowed in short communications.

Sequence of Preparation

1. The data files must be PC/Windows-compatible. The text should be prepared using standard software (Microsoft Word) format; do not use automated or manual hyphenation. Please do not include footnotes.
2. Use Times New Roman 12 point font for all text except for tables and figures where Times New Roman 10 font should be used.
3. Use 1 inch margins on top, bottom, left and right margins,
4. Every line on the text should be numbered.
5. Use double line spacing for body of text. For Abstract, Figures, Tables and References use single line spacing.
6. Place page numbers in the lower right hand corner of your manuscript.
7. Run "the spell check" and "grammar check" on the entire file before submission using either the UK English or French standard.
8. Avoid using abbreviations for the names of concepts. Use ordinary words for variable names – not code names or other abbreviations. Use the same name for a variable throughout your text, tables, figures and appendices. Names of organizations and research instruments may be abbreviated, but give the full name (with abbreviation in brackets) the first time you mention one of these.
9. References should take the following form: In the text, a reference identified by means of an author's name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author's name should be mentioned, followed by '*et al.,*'. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a' and 'b' after the date to distinguish the works. Examples: Abayomi (2000), Agindotan *et al.,*. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; Chukwura, 1987a,b; Tijani, 1995, 1993), (Kumasi *et al.,* 2001)

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Examples of References

- *Journal Articles:* Ouyang D, Bartholic J, Selegean J, 2005. Assessing sediment loading from agricultural croplands in the Great Lakes basin. Journal of American Science, 1(2): 14-21.
- *Books:* Durbin R, Eddy SR, Krogh A, Mitchison G, 1999. Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids. London, Cambridge University Press.

- *Chapter in a Book:* Leach J, 1993. Impacts of the Zebra Mussel (Dreissena polymorpha) on water quality and fish spawning reefs of Western Lake Erie. In Zebra Mussels: Biology, Impacts and Control, Eds., Nalepa T, Schloesser D, Ann Arbor, MI: Lewis Publishers, pp: 381-397.
- *Reports:* Makarewicz JC, Lewis T, Bertram P, 1995. Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. US EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.
- *Conference Proceedings:* Stock A, 2004. Signal Transduction in Bacteria. In the Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.
- *Thesis:* Strunk JL, 1991. The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior, Unpublished PhD thesis, Michigan State University, East Lansing, MI.
- *Web links:* Cerón-Muñoz M F, Tonhati H, Costa C N, Rojas-Sarmiento D and Solarte Portilla C 2004 Variance heterogeneity for milk yield in Brazilian and Colombian Holstein herds. Livestock Research for Rural Development. Volume 16, Article #20 Visited June 1, 2005, from http://www.lrrd.org/lrrd16/4/cero16020.htm

Illustrations
Please send the figures as separate files and do not import them into the text file. Put all tables, figures, diagrams and artwork on separate pages. Each figure, table, and bibliographic entry must have a reference in the text. References to tables and figures in the text should be by number and not to "table below" or "figure below". The Editor will place them in the appropriate place in the text of article during the final edit. Tables and figures should be numbered consecutively. Please submit the data for figures in black and white.

Abbreviations, Symbols and Nomenclature
All specifications must be stated according to the S.I. system. Concentrations of chemical solutions are to be given in mol/l. All other concentrations should be given in % (volume or weight). Any abbreviations of chemical, biological, medical or other terms should only be employed when it is certain that they are internationally known. The full name must be stated in brackets when the abbreviation is first used. Names of micro-organisms and zoological names should be italicized in the manuscript.

Ethical guidelines
BAHPA adheres to the below ethical guidelines for publication and research. Experimentation will only be published if such research has been conducted in full accordance with ethical principles. Manuscripts containing experimentations must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

1. When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort.
2. All studies using animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

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When you submit a revised version of your article in response to the referees' comments, you must accompany it with a detailed list of the changes made (ignoring typographical errors, but mentioning additional paragraphs, changes to figures, etc) suitable for transmission to the referee. Where changes have been made in response to the referees' remarks it is important to mention this and indicate where they can be found. You may also wish to send in a second copy of your article with the changes marked or underlined.

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